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CLARIFICATION OF MILK[1]

CHARLES E. MARSHALL AND E. G. HOOD

TOGETHER WITH ARTHUR N. JULIAN, S. G. MUTKEKAR AND MAX S. MARSHALL

Massachusetts Agricultural Experiment Station, Amherst, Massachusetts

That the clarifier is capable of performing certain functions in cleaning milk and modifying its fermentations is abundantly apparent to any close student of the process. Whether it has reached the acme of its powers is still to be demonstrated. In the present article it is the purpose of the writers to convey some of the causes for its influence upon microbial activities. It is not felt, however, that all of these have been determined.

In Bulletin 187, Massachusetts Agricultural Experiment Station, and in an article presented before the American Public Health Association[2] at New Orleans, a summation of what the clarifier does is obtainable and it need be repeated here only in a form to serve the purpose of presenting an analysis and proof. In order to classify the results without furnishing unnecessary and too many accumulated data, only illustrative experimental evidence will be offered throughout this article.

I. VARIATIONS IN THE MICROBIAL CONTENT OF MILK AFFECTING CLARIFICATION

It is a well established fact that milks differ very widely in their microbial content, consequently the reaction of milk to the clarifier must be variable. This has been set forth in one way and another throughout Bulletin 187 and especially is it set forth by the fact that the numbers of microörganisms eliminated are subject to great variations which are due to many factors. The fermentations incident thereto likewise are not uniform and are widely variable.

[1] This article will be published later by the Massachusetts Agricultural Experiment Station as Part II of Bulletin 187.

[2] Studies in the clarification of milk, by C. E. Marshall, E. G. Hood, S. G. Mutkekar, John Yesair and Max Marshall.

II. AN ANALYSIS OF SOME DIFFERENCES EXISTING BETWEEN UNCLARIFIED AND CLARIFIED MILK

1. Naked eye appearances. To the worker the differences between unclarified and clarified milk become more and more evident as he studies innumerable samples by contrast or comparison. Sometimes they are slight, sometimes very noticeable to the naked eye. They find their explanation in the evidence which follows.

2. Microbial evidences. It became necessary to utilize distinct species of microörganisms for satisfactory results because platings and isolations from unclarified and clarified samples of milk proved unsatisfactory in our hands in reaching an analytical determination of microbial values.

TABLE 1

Illustrative test; per cent of organisms eliminated by the clarifier

Oidium lactis	99+
Saccharomyces cerevisiae	99+
B. tumescens	66+
B. subtilis	47+
B. coli	35+
Strept. pyogenes	20+
Strept. lacticus	24+

An arrangement from the largest to the smallest forms of organisms will convey the significance of the action of the centrifugal force of the clarifier.

While these numbers may vary considerably, it is true that colonies or organisms in masses and large organisms are more readily cast out than small organisms, due probably to the relation of the surface area of the cell to the cell volume. This corresponds exactly with the work of any centrifuge.

3. Proteolysis.[3] There is every indication, too, that proteolysis takes place to a greater extent in unclarified than in clarified milk. This is attributable to the lactic direction given to fermentation by the clarifier. Particular attention will be given to this "directive influence" under "Causes for Differences" which follows

[3] These determinations were made by Mr. S. G. Mutkekar.

later. Out of the analyses of ten samples, the writers will offer only one inasmuch as all are very much the same. The Kjeldahl method was employed in the determinations.

4. Acidity. A difference in acidity has been recognized by several workers whose publications have been noted in Bulletin 187. There is a slight increase in the clarified samples. This increase is uniform and harmonizes with carbon dioxide findings which will be given later and with the lactic directive influence already suggested.

TABLE 2

Illustrative sample

AGE OF MILK	TOTAL N IN 100 CC. SERUM		PROTEIN PER 100 CC. SERUM		INCREASE OF SOLUBLE PROTEIN	
	A	B	A	B	A	B
days						
6	0.1317	0.1359	0.823	0.849		0.026
7	0.1345	0.1352	0.841	0.844		0.003
8	0.1303	0.1387	0.814	0.867		0.053
9	0.1310	0.1429	0.819	0.893		0.074
10	0.1275	0.1331	0.797	0.832		0.035
11	0.1303	0.1450	0.814	0.906		0.092
12	0.1331	0.1401	0.832	0.876		0.044
13	0.1457	0.1401	0.911	0.876	0.035	
14	0.1436	0.1471	0.897	0.919		0.012
15	0.1485	0.1583	0.928	0.989		0.061

A, clarified milk; B, unclarified milk.

Only one illustrative table will be inserted as representative of thirteen similar determinations made. The method of determination is included that the reader may give proper interpretation to the results.

Determination of acidity in milk serum. 100 cc. of milk were put in flasks which were held at temperature ranging from 12°–14°C. until the milk had curdled—usually three to five days. The milk was then filtered and serum obtained. An aliquot of 10 cc. of this serum was then pipetted into a flask and diluted to 100 cc. with distilled water. Two or three drops of phenolphthalein were then added and the titration accomplished by decinormal NaOH.

5. Gas production. The determination of the total amount of gas to be found in clarified and unclarified milk has not yet been attempted. However, the amount of carbon dioxide formed has been accurately measured by Prof. Arthur N. Julian who reports the results as shown in tables 4 and 5.

Methods used. In general, the method used in the following determinations of CO_2 production in clarified and unclarified milk is that employed by E. Truag of the Soils Department, Wisconsin Experiment

TABLE 3

Illustrative sample

DATE	AGE OF MILK	N/10 NaOH USED	
		B	A
1918	*days*	*cc.*	*cc.*
January 28	4	6.00	5.94
January 29	5	5.80	5.80
January 30	6	5.82	5.88
January 31	7	6.14	6.50
February 1	8	6.76	6.48
February 2	9	6.74	6.82
February 3	10	6.70	6.80
February 4	11	6.90	7.00
February 5	12	6.82	7.00
February 6	13	7.10	7.52
February 7	14	7.40	7.52
February 10	17	7.40	7.84
February 11	18	7.54	8.10
February 12	19	7.90	8.30

A, clarified milk; B, unclarified milk.

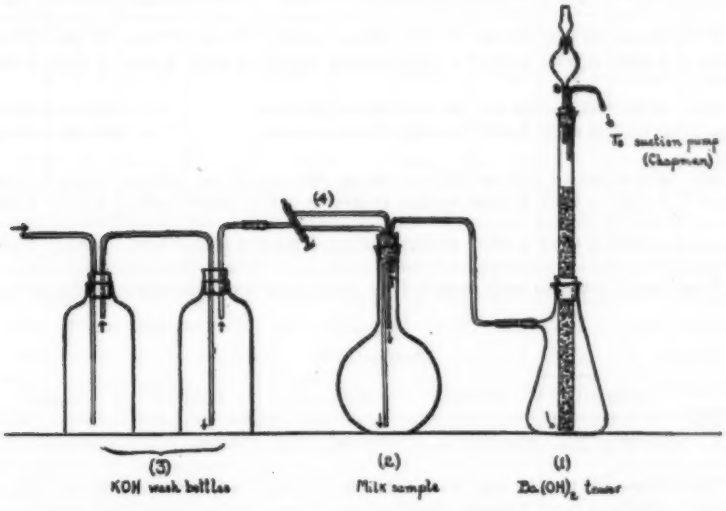
Station, in his work on CO_2 in soils. (J. Ind. and Eng. Chem., vii, no. 12, December, 1915).

Special adaptations to the problem in hand may be mentioned as follows, the accompanying diagram illustrating the arrangement of apparatus.

This apparatus was set up in duplicate, so that the clarified and unclarified samples of milk were receiving treatments as nearly as possible identical.

Two wash bottles containing strong KOH (*3*) and broken glass were employed to deliver CO_2-free air to the flask containing the milk

sample (*2*), then a two-way stopcock (*4*), one tube of which extended only to the surface of the milk in flask (*2*), while the second arm delivered air to the bottom of the flask. The first of these tubes (delivering air to the surface) was used in making the first two determinations of each experiment, representing the free evolution of gas; the arm extending to the bottom was employed in displacing the CO_2 gas held in solution, in making the third determination, approximating "total gas."



A third pair of wash bottles, identical with that in diagram, was employed in the preliminary filling of the Truag tower and suction flask (*1*) with CO_2-free air.

No attempt was made to determine how closely the "approximate total gas" of the third determination approached actual total gas, one and one-half hours' suction being chosen arbitrarily as a reasonable standard time for both samples, hence valuable only for comparative purposes.

The usual precautions were observed in the matter of uniformity of milk, safeguards against contamination from CO_2 of the air at all steps, etc.

The following table presents the results of ten experiments, five on raw commercial and five on raw certified milk, figures representing CO_2 in terms of grams per 1200 cc. milk employed.

TABLE 4

Market milk

DETERMINATION	SAMPLE I		SAMPLE II		SAMPLE III		SAMPLE IV		SAMPLE V	
	Clarified	Unclarified	Clarified	Unclarified	Clarified	Unclarified	Clarified	Unclarified	Clarified	Unclarified
1. 18–24 hrs.	24 hrs.	24 hrs.	24 hrs.	24 hrs.	24 hrs.	24 hrs.	24 hrs.	24 hrs.	27 hrs.	27 hrs.
	0.0108	0.0035	0.0103	0.0050	0.0024	0.0024	0.0132	0.0086	0.0130	0.0092
2. 42–48 hrs.	48 hrs.	48 hrs.	44 hrs.	44 hrs.	48 hrs.	48 hrs.			43 hrs.	43 hrs.
	0.0194	0.0176	0.0117	0.0035	0.0139	0.0123			0.0125	0.0051
3. 50–60 hrs.	60 hrs.	60 hrs.	53 hrs.	53 hrs.	60 hrs.	60 hrs.	40 hrs.	40 hrs.	53 hrs.	53 hrs.
	0.0755	0.0563	0.0290	0.0372	0.0161	0.0147	0.0227*	0.0205*	0.0257	0.0255
Totals	0.1057	0.0774	0.0510	0.0457	0.0324	0.0294	0.0359	0.0291	0.0512	0.0398

* Total at 40 hours to save sample from going over top; excessive gas formation.

TABLE 5

Certified milk

DETERMINATION	SAMPLE I		SAMPLE II		SAMPLE III		SAMPLE IV		SAMPLE V	
	Clarified	Unclarified	Clarified	Unclarified	Clarified	Unclarified	Clarified	Unclarified	Clarified	Unclarified
1. 18–24 hrs.	12 hrs.	12 hrs.	24 hrs.	24 hrs.	24 hrs.	24 hrs.	18 hrs.	18 hrs.	24 hrs.	24 hrs.
	0.0015	0.0011	0.0059	0.0018	0.0062	0.0020	0.0059	0.0015	0.0066	0.0020
2. 42–48 hrs.	36 hrs.	36 hrs.	48 hrs.	48 hrs.	48 hrs.	48 hrs.	42 hrs.	42 hrs.	42 hrs.	42 hrs.
	0.0090	0.0090	0.0152	0.0059	0.0266	0.0185	0.0145	0.0057	0.0134	0.0046
3. 50–60 hrs.	60 hrs.	60 hrs.	60 hrs.	60 hrs.	60 hrs.	60 hrs.	50 hrs.	50 hrs.	50 hrs.	50 hrs.
	0.0162	0.0279	0.0169	0.0172	0.0460	0.0583	0.0480	0.0526	0.1415	0.1078
Totals	0.0267	0.0380	0.0380	0.0249	0.0788	0.0788	0.0684	0.0598	0.1615	0.1144

From the results of Professor Julian the writers are led to interpret the increased amount of carbon dioxide in clarified milk as due to the stimulation given to lactic fermentation which has already been referred to and the reasons for which will appear in connection with other considerations.

6. Methylene blue reduction. The reduction of methylene blue is so little understood that to draw any particular conclusions is precarious. Some have claimed that clarified milk has reduced methylene blue more quickly than unclarified milk and others that unclarified acts more rapidly. The illustrative tests inserted will indicate that the unclarified milk has reduced methylene blue more rapidly than the clarified although constancy does not exist throughout. The limited number of tests presented as illustrative furnish a fair notion of the general results secured.

Method employed for reduction of methylene blue. Methylene blue: 5 cc. concentrated alcoholic solution and 195 cc. distilled water. 2 cc. of this solution is added to 50 cc. of milk and the mixture well shaken until the color is uniform. Better to make this mixture in sterile flasks and then pour into the test bottles. These bottles are then kept in the incubator at 37°C. and the changes noted from time to time.

7. Character of curd.—It has been convincing to ascertain in a large percentage of cases that the casein obtained by filtration from unclarified and clarified milk followed a distinctive course in decomposition. The casein deposit from the unclarified milk produced molds abundantly and seemed to undergo proteolysis readily and rapidly; that from the clarified milk seemed much freer from molds and proteolysis and followed the course as witnessed in cheese curd when acid has been developed. This bears testimony to the starter-effect of the clarified which has been reverted to in this discussion quite frequently.

In securing the above curds, the milk samples before and after clarifying were set aside in a suitable place. In our work a room having a constant temperature of about 17°C. has been used. After the milk samples had curded, they were carefully filtered through sterile filter paper. The precipitated curd, covered over, was held on the paper in the funnel and the changes watched.

This procedure proved to be one of the most striking crude methods of noting the differences between unclarified and clarified milk. If care is exercised, it is a method which may be easily utilized without laboratory facilities.

TABLE 6

Illustrative tests; reduction of methylene blue; certified milk

NUMBER OF TEST	DATE	SAMPLE	AGE OF MILK	TEMPERATURE OF HOLDING	TIME OF STARTING REDUCTION		TIME OF COMPLETE REDUCTION		TOTAL TIME OF REDUCTION		BACTERIA PER CUBIC CENTIMETER
					Hours	Minutes	Hours	Minutes	Hours	Minutes	
				deg. C.							
I	4/15	A	Once	20–22	6	37	3	5	9	42	3,600
		B	Once	20–22	6	0	3	0	9	0	5,500
	4/16	A	24	20–22	0	49	0	34	1	23	8,000,000
		B	24	20–22	1	23	0	22	1	45	7,200,000
	4/17	A	48	20–22	1	15	0	30	1	35	60,000,000
		B	48	20–22	1	0	0	8	1	8	84,000,000
II	4/20	A	Once	20–22	6	12	2	50	9	2	4,800
		B	Once	20–22	5	45	2	30	8	15	5,900
	4/21	A	24	20–22	1	58	0	19	2	17	7,300,000
		B	24	20–22	1	18	0	23	1	41	9,800,000
	4/22	A	48	20–22	0	17	0	11	0	28	
		B	48	20–22	0	21	0	10	0	31	
III	4/23	A	Once	20–22	4	45	0	54	5	39	250,000
		B	Once	20–22	4	30	1	0	5	30	400,000
	4/24	A	24	20–22	0	22	0	3	0	25	63,000,000
		B	24	20–22	0	15	0	5	0	20	121,000,000
	4/25	A	48	20–22	0	32	0	10	0	42	189,000,000
		B	48	20–22	0	28	0	10	0	38	145,000,000
IV	5/2	A	Once	20–22	7	15	0	30	7	45	4,000
		B	Once	20–22	6	10	1	15	7	25	8,000
	5/3	A	24	20–22	0	58	0	12	1	10	20,000,000
		B	24	20–22	0	47	0	8	0	55	12,000,000
	5/4	A	48	20–22	0	57	0	19	1	16	49,000,000
		B	48	20–22	0	33	0	26	0	56	57,000,000
V	5/10	A	Once	20–22	11	15	4	15	15	30	1,600
		B	Once	20–22	10	45	4	0	14	45	4,100
	5/11	A	24	20–22	4	15	3	22	7	37	320,000
		B	24	20–22	4	0	3	0	7	0	450,000
	5/12	A	48	20–22	1	40	0	15	1	55	21,000,000
		B	48	20–22	0	25	0	3	0	28	46,000,000
VI	5/16	A	Once	20–22	4	40	1	25	6	5	1,020,000
		B	Once	20–22	4	15	1	50	5	55	1,520,000
	5/17	A	24	20–22	0	7	0	17	0	34	219,000,000
		B	24	20–22	0	4	0	3	0	7	320,000,000
	5/18	A	48	20–22	*						
		B	48	20–22							

A, clarified milk; B, unclarified milk.

* Milk coagulated.

The laboratory tests indicate that clarified and unclarified milk may be differentiated. The surface of the unclarified sample should reveal an abundant growth of molds while the clarified sample will appear quite free from molds. If this is true of molds, it is easily understood how yeasts and large microorganisms have been eliminated in clarifying. The influence exerted by these organisms upon milk may be measured in a degree by the study of association.

These tests with the curds and with the samples of unclarified and clarified milks are visible, striking and convincing, although furnishing only a crude interpretation.

III. THE LEADING CAUSES OF SOME OF THE DIFFERENCES BETWEEN UNCLARIFIED AND CLARIFIED MILK

1. Selective action. By selective action is meant the power of the clarifier to pick out the heavier organisms in greater percentage than the lighter organisms. This power has been reverted to in Bulletin 187 and again at the beginning of this paper in which the rate of elimination of various organisms by the clarifier is given. In this respect the clarifier corresponds closely to a centrifuge.

Using an International Instrument Company's No. 1-B centrifuge with a speed of 2250 revolutions per minute and suspending the microörganisms in milk the following results were secured.

Comparing the work of the centrifuge with that of the clarifier above, it would be approximately true to say that the centrifuge running ten to fifteen minutes gives about the same results as the clarifier gives running at 8000 revolutions per minute and in which the passage of milk is effected in about seven seconds.

The clarifier has demonstrated its efficiency in casting out dirt particles which have a specific gravity greater than milk. The larger microörganisms apparently respond to its action very readily as is shown in table 1. This is due to the specific gravity and likewise to the relation of surface to cell-volume. The smaller the organism, the greater the extent of surface is

to its cell-volume although the density of the cell-contents may be the same as in the larger organism or may be different. It is also a matter of common observation among microbiologists that microörganisms are not constant in their density.

TABLE 7

Illustrative tests

TIME	MICROÖRGANISMS		
	B. subtilis per loop	*Strept. lacticus* per loop	*Oidium lactis* spores per loop
minutes			
At start	3561	164	180
5	1017	121	3
10	349	104	1
15	84	84	1
20	48	73	
25	13	49	
30	8	45	
Per cent removed....	99.8	72	100, at end of 20 minutes

TABLE 8

	PER CENT OF TOTAL ELIMINATED IN		
	First Clarification	Second Clarification	Third Clarification
Sacch. cerevisiae.....	99.2	99.6	99.9
Strept. lacticus.....	20.0	55.1	85.1

Comparing two organisms, *Saccharomyces cerevisiae*, a large organism, and *Streptococcus lacticus*, a small organism, in whole milk after passage through the clarifier three times will give some idea of the relative results. The removal of such organisms as *Oidium lactis*, yeasts and large bacteria has a decided tendency to disturb the germ-equilibrium which, in the case of milk, results in the lactic organisms, which are very small and do not colonize readily, dominating the field and thus giving rise to quickened lactic fermentation. When operating together with two other factors, associative influences and distribution, which

will now be considered, it is readily seen that a combination of forces is operative in a clarifier, each one of which has some influence, although difficult to determine quantitatively.

2. Associative influences. It will suffice, in this connection, to confine ourselves to a single study which will set forth our ideas, although many phases of this particular subject has been investigated.

Preparation. Forty 300-cc. flasks containing 100 cc. of litmus skimmed milk each were made ready by the usual methods. The milk was sweet (no acid could be detected) skimmed market milk and of the same lot. These flasks were arranged in duplicate series. Two flasks were held as sterile checks and two used for *Strept. lacticus* control. Three series of six flasks each in duplicate were treated as indicated in table 9. The inocula consisted of a culture of *Strept. lacticus* diluted so that each drop from a Roux pipette contained 200 organisms; of *B. subtilis* diluted in the same manner but each drop contained only 25 organisms; of *Oidium lactis* spores diluted in the same manner but each drop contained 500 spores. The flasks were arranged and inoculated according to the following scheme with the results indicated opposite each series.

Under "selection" it is evident that there is a tendency to disturb the numerical relationships or equilibrium of microorganisms present in milk or any other fluid serving as a menstruum for fermentation. This disturbed relationship, therefore, must result in an altered or modified fermentation. For instance, it is easily demonstrated that the presence of *B. subtilis* in association with *Strept. lacticus* hastens the action of the latter. It is also easily demonstrated and well known that with the rapid development of *Strept. lacticus* in the presence of *Oidium lactis*, the growth of *Oidium lactis* is hastened. Further, it can be easily demonstrated that the growth of *B. subtilis and Strept. lacticus* stimulates the *Oidium lactis* beyond that of the *Strept. lacticus* alone. Now if a centrifuge or clarifier is introduced in a mixed culture of *Strept. lacticus, B. subtilis and Oidium lactis*, in milk, the *Oidium lactis* is thrown out to such a degree that when *Strept. lacticus* develops only a colony here and there will be found; *Oidium lactis* growth becomes practically negligible.

TABLE 9

Illustrative associative study

	FLASK NUMBER	INOCULA	RESULTS
Check Series I	1	Sterile—none	Remained sterile
	2	Sterile—none	
Strept. lacticus, Series II		*Strept. lacticus*	
	1	1 drop	Curded in 72 hours with litmus reduction normal
	2	1 drop	
Strept. lacticus + *B. subtilis*, Series III		*Strept. lacticus* *B. subtilis*	
	1	1 drop + 1 drop	Whole series finished curding in 53 hours. Litmus changes slightly altered. Series flask No. 6 curded in 51 hours or 2 hours before series flask No. 1. Curding proceeded from 6 to 1
	2	1 drop + 4 drops	
	3	1 drop + 8 drops	
	4	1 drop + 12 drops	
	5	1 drop + 16 drops	
	6	1 drop + 20 drops	
Strept. lacticus + *Oidium lactis*, Series IV		*Strept. lacticus* *Oidium lactis*	
	1	1 drop + 1 drop	Whole series curded together in 72 hours same as Series II. *O. lactis* does not affect cultures; does not grow much before acid forms. Growth of *O. lactis* normal and vigorous. Destruction of acid by *O. lactis* was normal. Litmus changes followed lactic fermentation as in Series II till *O. lactis* reduced it almost permanently.
	2	1 drop + 4 drops	
	4	1 drop + 8 drops	
	4	1 drop + 12 drops	
	5	1 drop + 16 drops	
	6	1 drop + 20 drops	

TABLE 9—*Continued*

	FLASK NUMBER	INOCULA			RESULTS
		Strept. lacticus	*B. subtilis*	*Oidium lactis*	
Strept. lacticus + *B. subtilis* + *Oidium lactis*, Series V	1	1 drop +	1 drop +	1 drop	Whole series curded in 56 hours or 3 hours later than Series III. Series flask No. 6 curded in 53 hours or 3 hours earlier than series flask No. 1. Curding proceeded from 6 to 1. Growth of *O. lactis* more rapid and more vigorous than in Series IV. Destruction of acid by *O. lactis* also more rapid than in Series IV. Litmus changes same as in Series IV but much more pronounced.
	2	1 drop +	4 drops +	4 drops	
	3	1 drop +	8 drops +	8 drops	
	4	1 drop +	12 drops +	12 drops	
	5	1 drop +	16 drops +	16 drops	
	6	1 drop +	20 drops +	20 drops	

One drop *Streptococcus lacticus* = 200 organisms; 1 drop *Bacillus subtilis* = 25 organisms; 1 drop *Oidium lactis* = 500 organisms.

Room temperature 17° to 25°C. maintained.

Further, should *B. subtilis* be removed to such an extent that its stimulating influence upon *Strept. lacticus* and *Oidium lactis* is submerged, then it can readily be seen that the fermentation produced by *Strept. lacticus* and *Oidium lactis* is retarded. In such a combination or mixed culture in milk as just named, *Strept. lacticus* is eliminated by the clarifier in a much less degree, 24 per cent, than is either of the other organisms, *Oidium lactis*, 99 + per cent, of *B. subtilis*, 47 + per cent. The result will be that *Strept. lacticus* left behind is likely to act very much as a starter or become actually conspicuous by its retention and fermentation. The other two organisms, *B. subtilis and Oidium lactis*, lose a large share of their significance.

Such combinations of microörganisms or mixed cultures can easily be followed to a point where the individualities of the organisms sink beyond recognition; in other words, associative influences may be followed as long as the species present manifest to the observer their individual action; then the analysis, or synthesis as the case may be, loses its clear definition and the observer becomes confused, no individuality of species can be determined. This evidence, however, has its bearing in connection with some of the results obtained from the use of the clarifier with certified or market milk,—a modification of the fermentation is evident in the majority of samples but this modification is extremely difficult to interpret. Differences in fermentation are detected many times between the unclarified and clarified milk, yet so complex and so variable are the conditions and the germ-content that an understanding must be through indirect channels and influences. So difficult, in fact, is it to determine these elemental differences, or factors involved, in terms of definite, positive and known data that it seems necessary at present to resort to this inferential study of selection and association if a clear idea is to be gained of what actually transpires in the use of the clarifier.

3. Distributive action. It has been possible to demonstrate the distributive action of the clarifier in several ways.

a. The increase in the number of microörganisms in market milk after clarification as revealed in Bulletin 187 is of common experience. In fresh or certified milk where colonization is not permitted to take place so freely the tendency is toward reduction in numbers.

b. When *B. coli* is placed in glucose agar and the agar passed through the clarifier, the colonies resulting from the increased number of foci and the formation of gas bubbles at these points give a graphic illustration of what has taken place in the clarifier—a colony apparently is broken into innumerable fragments.

c. If a little oil is placed in water and the mixture passed through the clarifier, the mixture becomes a very fine emulsion.

All of these experiences confirm the influence of the clarifier in causing the breaking up of colonies or groups of microörgan-

isms in milk and causing their distribution throughout the mass.

4. Aeration. Air is taken into the clarifier bowl and mixed with the milk. It also finds its way in when impinging against the surface of the receiving pan as it emerges from the bowl. This is not a constant amount but the ratio as determined is about 7 to 3.

If milk is thoroughly aerated while clarifying, it is easily understood how the growth of microörganisms is affected. The action in clarification, so far as can be detected, favors lactic fermentation.

5. Unknown influences. The writers feel that they have not yet encompassed all of the agencies at work in the clarifier. This attitude results from certain manifestations which the writers have not yet been able to attack effectively or are so concealed that they frustrate attempts.

SUMMARY

1. The clarifier by its action modifies the fermentations of milk by its influence upon the germ-content.

2. These differences in the fermentation of clarified and unclarified milk are made manifest by

(1) Alterations visible to the naked eye. These are not transferable to paper but are due to the factors in part indicated below.

(2) Microbial evidences such as the growth of *Oidium lactis* upon the surface of the milk or the lactic fermentation.

(3) Proteolysis.

(4) Acidity.

(5) Gas production.

(6) Methylene blue reduction.

(7) Character of curd.

3. The causes for these differences cited under 2 are

(1) Selective action of the clarifier.

(2) Associative influences resulting from disturbed germ-content.

(3) Distributive action of clarifier.

(4) Aeration effected by clarifier.

(5) Causes at present unknown or undeterminable.

THE RELATION OF AGE OF DAM TO OBSERVED FECUNDITY IN DOMESTICATED ANIMALS

I. MULTIPLE BIRTHS IN CATTLE AND SHEEP[1]

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INTRODUCTION

The fact that the present paper is concerned with fecundity as affected by the age of the dam and that the term fecundity is not always employed as indicating the same function, necessitates a definition of the term. This necessity is further emphasized by past usage in the literature on the physiology of reproduction and in stock journals, where the words fecundity and fertility are often used interchangeably.

The literature on the physiology of reproduction not only shows the words fertility and fecundity used interchangeably, but the same is true of the terms fertilization and fecundation. Dalton (1875) and Flint (1876) employed fecundation as referring to the union of egg and sperm. Others as Draper (1856) and Dorland (1896) used either term when referring to this process, while later physiologists, as Landois and Sterling (1889), Howell (1901), Marshall (1910), Starling (1912) and Halliburton (1917) have given preference to the term fertilization. The similarity in the meanings of these two terms is further illustrated by their definitions as given by Dorland's Medical Dictionary (1919). Fecundation is defined as "Impregnation or fertilization," while fertilization is "the act of rendering fertile; fecundation." Furthermore, formal definitions of fertility and fecundity, as given, for example, by the Century Dictionary, treat them as practically

[1] Papers from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin; No. 24. Published with the approval of the Director of the Station.

synonymous. Fecundity is defined as "Fruitfulness; the quality of propagating abundantly; particularly the quality in female animals of producing young in great numbers." Turning to the definition of fertility we find: "The state of being fertile or fruitful; the quality of producing in abundance; fecundity; productiveness. . . . . ."

In common usage fertile is employed as the antithesis of sterile, that is an animal is said to be fertile when capable of producing offspring, while on the other hand a female is called fecund if she produces large numbers of offspring. The four terms fertility, fecundity, fertilization and fecundation refer then to one process, namely the production of offspring. But before offspring may result an egg and spermatozoon must have fused, and before this can occur ova must be liberated from the ovary. Ovulation occurs periodically and may continue to do so quite independently of the production of young. We have then two conditions, first, the discharge of eggs from the ovary and second the union of egg and sperm, normally resulting in the production of young. Pearl and Surface (1909, p. 81) in their work with fowls differentiated these two conditions, assigning the term fecundity to the former and fertility to the latter. Thus fecundity is "the innate potential reproductive capacity of the individual organism, as denoted by its ability to form and separate from the body mature germ cells. Fecundity in the female will depend upon the production of ova and in the male upon the production of spermatozoa." Fertility as defined by these authors is "the total actual reproductive capacity of *pairs* of organisms, male and female, as expressed by their ability when mated together to produce (i.e., bring to birth) individual offspring."

This definition of fecundity relates to *actual* fecundity, or the actual number of eggs discharged from the ovary of a female regardless of the production of offspring. Mention may be made of a second type of fecundity, namely *potential*, there being more potential ova present in the ovaries than can, because of physical limitation, be discharged in the lifetime of an individual. Poten-

tial fecundity is difficult, if not impossible, of measurement.[2] Actual fecundity is possible of determination in birds (except for resorbed eggs), but not in mammals, since the eggs of mammals are microscopic in size and hence are lost to record unless they undergo further development. Since, therefore, actual fecundity cannot be directly determined in mammals, it becomes necessary to express fecundity in them in terms of fertility as defined by Pearl and Surface. This may accordingly be designated as *observed* fecundity.

It would be of importance to determine, if possible, the degree of accuracy which may be obtained in expressing fecundity in this way. So far as the authors are aware there has been no direct attempt to make this determination. Certain embryological studies, however, supply observations which serve to give some idea of the degree of correspondence between actual and observed fecundity. For example, there are cases in which comparison may be made between the number of ripe follicles in the ovaries of any female and the average number of young normally produced in a single litter. Honoré (1900) in his work on the rabbit found five follicles about to rupture in one ovary of a female which had been bred a few hours previous to examination. No mention is made of the other ovary but, if we assume that as a matter of chance it had the same number of ripe follicles as the one examined, the total number in both ovaries would be ten, which is in excess of the average number of young per litter in rabbits, for according to Marshall (1910, pp. 588 and 591), who takes Spencer and Darwin as his authorities, six or seven is the average litter size in this species. A few similar data on the bat are available from the work of Van der Stricht (1901). An examination of the ovaries of several individuals revealed one, two or three ripe follicles. Taking Marshall (1910, p. 587)

[2] Pearl (1912) attempted to measure potential fecundity in the domestic fowl by counting the oocytes visible to the naked eye in the ovaries of several hens. Such counts, made for seventeen different individuals, showed each ovary to contain an average of 1676 oocytes. Pearl, however, states that this number is a minimum one, since there is a large number of oocytes invisible to the naked eye, but nevertheless present in the ovary and capable of further growth and development.

as authority again, the bat as a rule produces but one individual at a single parturition, so again there are more ripe follicles than offspring normally produced. These examples are inserted here merely to illustrate a possible way of studying actual fecundity in mammals, and as giving an indication that observed fecundity is not a complete measure of actual fecundity.

Other embryological studies make it possible to compare the number of corpora lutea with the number of embryos in the uterus. Sobotta (1896) in his studies on the mouse found three corpora lutea in one ovary of a female and three embryos in the corresponding horn of the uterus, while the other ovary showed two corpora lutea, but no mention is made of embryos in the horn of this side, so presumably there were none. Later the same author (1897) examined the ovaries of eight rabbits at different periods of gestation and found the following conditions. The largest number of corpora lutea in the ovaries of any one rabbit was twelve, with nine embryos in the uterus, while the smallest number of corpora lutea in any of the eight rabbits was four, with three embryos. In every case the corpora lutea exceeded the embryos in number. The total number of corpora lutea in all the rabbits was fifty-nine, an excess of eighteen over the total number of embryos, which was forty-one. Only 70 per cent, therefore, of the corpora lutea were represented by embryos.

There is still another possible method of comparison, namely, between the number of corpora lutea and the number of offspring normally produced by any female in a single litter. Data on this point are more extensive, but only the most important of these will be presented. Marshall (1904) in his study of the œstrous cycle in sheep examined the ovaries of fifty-five females. Forty-two individuals showed one ruptured follicle in one ovary or the other, twelve had two ruptured follicles, one in each ovary or both in one, while one individual had three ruptured follicles, two in one ovary and one in the other. Summarizing this Marshall says (p. 74): "Thus in less than 24 per cent of the cases examined where ovulation had occurred, was more than one follicle found to have discharged." He concludes: ". . . . in view of the percentage of follicles discharged (as noted) being

scarcely, if at all, in excess of the usual percentage of lambs produced for the breeds in question, the converse of this statement is most probably also generally true." In other words about 76 per cent of the fifty-five ewes showed but one ruptured follicle, which indicates that practically three-fourths of these ewes would have produced but one offspring. According to Marshall this condition coincides with the average number of lambs actually produced singly by the breeds under consideration.[3]

Corner (1915) in examining 128 pairs of ovaries taken from pregnant swine, found from one to sixteen corpora lutea in both ovaries, eight being the most common number. According to the author's records six was the most frequent number of pigs per litter, while the range was from one to ten. The number of corpora lutea accordingly exceeded the number of pigs per litter.

It is obvious from the foregoing embryological studies that there is some discrepancy between actual and observed fecundity. This may be accounted for in part by certain physiological conditions connected with ovulation and reproduction. For instance, fecundity in ovulating virgin females is undeterminable, but is presumably higher than in pregnant females, since ovulation rarely if ever occurs during pregnancy. Furthermore females may not be bred regularly, and often when they are bred they fail to conceive. The fact that the eggs discharged at any ovulating period may not all be fertilized has just been demonstrated. On the other hand eggs may be fertilized, attain some development, and then degenerate. In either case these eggs are lost to record. One of the causes of this degeneracy is genetic lethals, as illustrated by the death *in utero* of the homozygous yellow mouse (Ibsen and Steigleder 1917). Other points might perhaps be mentioned, but these will suffice to show that there are a number of factors causing discrepancies between actual fecundity in mammals and that which is observed.

[3] Data presented by Bell (see p. 270 of this paper) and the present authors (p. 284) show the percentage of ewes producing single and twin lambs to be about equal, that is only 50 per cent of the ewes considered in these records drop single lambs.

In spite of these disturbing factors the discrepancy between observed and actual fecundity is not as great as might be supposed. This is of importance since the latter is the only practical measure of fecundity in mammals.

FECUNDITY AS AFFECTED BY AGE

Experiments and observations on multiparous animals have shown that when breeding is unrestricted the frequency of production of litters normally increases with the age of the female, at least up to a certain point. Records taken by Miss King (1916), for example, on the reproductive rates of seventy-six female rats revealed the fact that they produced fewer litters at the mean ages of 90 and 120 days than they did at 150, 180, 210 or 240 days. This point cannot readily be determined for the normally uniparous domesticated animals, in part because some of them ordinarily breed only at definite seasons, and even more because their breeding is not unrestricted, but is regulated by man to meet his convenience.

Records on multiparous animals further indicate that the average size of litter also increases with the age of the female until a fairly advanced age is attained. The data which show a similar physiological tendency in uniparous animals and man are rarely if ever interpreted as increase in litter size. This is because the occurrence of multiple births is relatively uncommon and hence in all cases the average litter size is only slightly in excess of one. This is particularly true in cattle and horses; in sheep, where the number of multiple births may be as great as 50 per cent, the average litter size may correspondingly be as high as 1.5, or even higher if any considerable proportion of the multiple births are triplets. Where the multiple births are practically all twin births, as in cattle, the "average litter size" is expressed by 1 plus the percent of multiple births. Average litter size for any age may readily be deduced from any expression of the proportion of multiple births to all births at that age.

In the following pages a review is first given of literature on the relation of age to fecundity in mammals, and this is followed by an analysis of data relating to cattle and sheep.

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SUMMARY OF LITERATURE

Data relating to the subject of this paper pertain chiefly to domesticated rodents (rats, rabbits, and guinea-pigs), the larger farm animals (swine, sheep and cattle) and man. Records on the first class of animals have been taken directly from experiments; most of those on the second class from Herd Books; while the few on man are collected from hospital records.

Miss King (1916) draws the following conclusions from the breeding records of seventy-six rats: (1) ". . . . a female rat reaches the height of her reproductive capacity when she is about seven months of age. This age represents also the median point in the animal's breeding career. That is one-half the total number of her offspring are produced by the time she has reached this age and one-half are produced afterwards" (p. 273). (2) The largest sized litters were produced by females of a mean age of 120 days. This was determined by averaging the litters produced by females of given ages. (3) The second litter is on the average the largest of the five or six usually produced by the rat. These last two points are practically the same in their significance, for, since rats produce their first litters at about a mean age of ninety days, they could not produce the second one till they were approximately 120 days old, the mean age at which the largest litters are cast.

Observations made by P. G. Bailey on the size of litters of rabbits are quoted by Hammond (1914, p. 266). The sizes of the litters are considered from the order of their occurrence, rather than the actual age of the mother. No mention is made

of the number of litters upon which the following figures are based. So far as they go, these findings show the second litter to be the largest, as in the rat.

Litter sequence	*Average size of litter*
First litter	5.58±0.32
Second litter	7.25±0.41
Third litter	7.08±0.38

Minot (1891) found that the number of guinea-pigs in a litter increases with the age of the mother up to 15½ months, when the greatest number of offspring are produced. Table 1, taken from Minot, shows in general an increase in litter size with the age of the female. The largest number of "first" litters cast contain one or two individuals and the largest number of "second" litters, three individuals. The suggestion is made that females might be kept in virginity for several years and then bred, in

TABLE 1

Sizes of successive litters in guinea-pigs (Minot)

NUMBER OF LITTER	1	2	3	4	5	6	7	8
First	16	25	9				1	
Second		9	13	6	1			
Third		6	6	3				
Fourth			2	2				

order to compare the size of their litters "with those of young primiperæ and of multiperæ of their own age."[4]

Marshall (1910, p. 590) makes a statement to the effect that dogs have fewer puppies in early litters than in later ones. The same is said to be true of the bear and elk, though no data are given to substantiate these statements.

More extensive observations relating to this subject have been made on swine and sheep than on any other farm animals, probably because of the importance of the matter to livestock breeders and the facility of obtaining data on these particular animals. Rommel (1907) has considered the relation of the

[4] An experiment to test this theory was started by Dr. H. L. Ibsen at this Station and is being continued by him at the Kansas State Agricultural College.

age of sows to the number of offspring produced. He found from a study of the Poland China Herd Books that the size of litters of sows increases with their ages as follows (p. 204):

Age of sow	*Average size of litter*
1 year	6.65
2 years	7.56
3 years	7.88
4 years	8.28
5 years	8.40

The above figures show an increase in litter size from one to five years. However five years cannot be definitely stated as the age for maximum litter-size production, because the data for subsequent ages were too few to be significant. The average size for all litters is 7.39.

Rommel points out that breeders should not dispose of their brood sows after producing one or two litters, but should keep them in order to obtain larger numbers of offspring.

Frölich and Georgs (1911) record the size of litter for a white breed of German swine (Edelschwein). Their results are obtained from the breeding histories of forty-six sows producing 1739 pigs. Since these authors consider the size of litters in relation to their sequence, rather than the actual age of the mother, the data which follow are not directly comparable to Rommel's and Minot's on swine and guinea-pigs.

Litter number	*Litter size*
1	7.98
2	8.74
3	8.26
4	7.72
5	7.54
6	9.00
Average for six litters	8.22

The maximum size is said by the authors to be reached in the second litter, the large recorded number in the sixth litter being attributed to the fact that the less fertile sows have been eliminated, only the most fertile ones being retained. A comparison of this material with that of Rommel's reveals certain

differences. It is unreasonable to suppose that the sows reported by Rommel were only just producing their second litters at five years, and it is also improbable that the sows considered by Frölich and Georgs were five years old when they gave birth to their second litters. These discrepancies may be attributed in part at least to two factors: (1) Rommel's results were obtained from a larger number of sows than were those of Frölich and Georgs; (2) there may be breed differences.

Machens (1915) also records the sizes of litters produced by sows of various ages in another German breed of swine. His data, based on a study of 362 litters, do not agree with either those of Rommel or Frölich and Georgs, since Machens finds the fourth litter the largest produced by this breed of sows. The average sized litter for sows of all ages is 9.56, which is higher than the averages for the other two breeds of swine just discussed.

In 1909 Mumford (1917) started an experiment with swine, which bears directly on the present subject. He obtained litters from immature sows (four or five months old), half mature sows (eighteen months old) and mature sows (twenty-four to thirty months). The results up to 1913 showed the average size of the litters for the three classes as follows, immature 4.8, half mature 6.3 and mature 6.5.

In the twenty-eighth annual Report of the State College of Washington (1919) data are presented on the average size of litter for sows of various ages as follows: 16 one year olds 6.12, 17 two year olds 7.7, 10 three year olds 7.8, and 4 four year olds 7.9. Records on older females are not given.

Hammond (1914) examined the ovaries of eighteen young sows, killed just after the heat period, and found the average number of corpora lutea in both ovaries to be 14.3 ± 0.39, while the ovaries of nine older sows showed 19.77 ± 1.26 corpora lutea.

The foregoing results on swine differ somewhat in details, but indicate that in general older sows tend to produce more eggs than younger ones, and consequently larger litters. (Note page 290.)

Heape (1899) found that in the Dorset Horned breed of sheep fewer twins were dropped by younger than older females. Only a bare statement is made, no data being presented.

Carlyle and McConnell (1902) show from records taken on the flock at the Wisconsin Station, that the percent of twin and triplet births increases with the age of the ewe in mutton breeds. The largest percentages of single, twin and triplet births are produced respectively by two, five and six year old ewes. Later data, reported by Humphrey and Kleinheinz (1907) from the same flock, confirm the findings of Carlyle and McConnell as to the ages at which ewes produce the largest percent of single and triplet births, but they found that the highest percent of twin births was produced by four year old instead of five year old ewes.

Pearl (1913) gives the unusual breeding record of a single ewe for nineteen years. She produced single lambs at one and two years of age, twins at three years, from four to nine years triplets for each year, twins again for each year from her tenth to fifteenth and singles for her sixteenth and seventeenth years. The maximum fecundity occurs at 7.34 years. This single case illustrates both the increase and subsequent decline in litter size with the advancing age of the female.

Alexander Bell (1904–1912) in his experiments with multi-nippled sheep did not find them, as he expected, more fertile than their normally nippled sisters. However, in his 1912 report Bell concludes that all the older ewes had a greater tendency to produce multiple births than the younger females. Thirty-six per cent, apparently, of all lambs born of young ewes were twins, while 60 per cent of the lambs born in the spring of 1912 of three year old ewes were twins. Popenoe (1914) quotes from another paper of Bell's, "ewes four, five and six years old yield a larger percentage of twins than younger or older ewes." These data of Bell's and the preceding findings on sheep lead to the same general conclusion as did those on swine, namely, that the tendency to produce larger litters is greater among older than younger females and may be followed by a subsequent decline in animals kept to a greater age.

Data on the frequency of multiple births in cattle are meager, though a great many items on the reproductive records of individual cows might be gleaned from the stock journals. The

authenticity of these scattered notes may in some cases be questioned. Nevertheless we will present three cases as given by Pearl (1912). The first, taken from the National Livestock Journal (1879), states that a certain cow gave birth to twins

TABLE 2

Breeding history of cow owned by Mr. Starret (Pearl)

YEAR	AGE	NUMBER OF CALVES PRODUCED
Born		
1900		
1902	2	1
1903	3	1
1904	4	1
1905	5	2
1906	6	2
1907	7	3
1909	9	1
1910	10	3
Total		14

TABLE 3

Breeding history of cow cited by McGillivray (Pearl)

YEAR	AGE	NUMBER OF CALVES PRODUCED	REMARKS
Probably born 1840			
1842	2	1	This was the cow's first calf
1843	3	3	All lived to be adults
1843	3	4	One died (7 calves in one year)
1844	4	2	Lived to maturity
1845	5	3	Lived to maturity
1846	6	6	All died prematurely
1847	7	2	Lived to maturity
1848	8	4	
Total		25	

three times in succession and then to triplets. Pearl next gives the breeding history of a cow belonging to a Mr. Starrett living near Waldoboro, Maine. This history is reproduced in table 2. It shows that the cow reached the high point of her reproductive

activity at seven years, when she produced triplets. The fact that she produced triplets again at ten years would seem to indicate that the high fecundity was being maintained at least to that age. A third case (table 3) presented by Pearl is taken from McGillivray's "Manual of Veterinary Science" (1857). This shows a more or less regular increase in the number of young produced by a cow up to the age of six years, when she is claimed to have aborted six embryos.

The ages at which various women produced 756 pairs of twins as reported by Duncan (1866) are shown in table 4. The records

TABLE 4

Occurrence of twins in 1512 parturient women (Duncan)

	AGES							
	15–19	20–24	25–29	30–34	35–39	40–44	45	Total
Collins	3	53	76	71	28	8	1	240
M'Clintock	2	23	45	41	17	1		129
M'Clintock and Hardy	1	20	34	26	12	2		95
Chiari Braun and Spaeth	1	25	36	26	4	2		94
Statistics of 1855	3	28	46	58	52	11		198
Total	10	149	237	222	113	24	1	756

were collected by the author from various sources and recorded under the ages of the mothers, which are arranged in five year periods. As Duncan (p. 73) says: "This table shows that in the general population it so happens that the number of twins born increases with the age of the mother until the age from twenty-five to twenty-nine inclusive is reached and that after this age is passed the number of twins born regularly diminishes." Duncan quotes from Dr. Collins to the effect that the mean age of 16,385 parturient women is twenty-seven, while that for 240 women bearing twins is twenty-nine.

MULTIPLE BIRTHS IN CATTLE

Source of data

In undertaking the present study the American Herd Books of the two beef breeds, Hereford and Aberdeen-Angus, seemed to offer the largest amount of desirable data. In the first place beef breeds were chosen instead of dairy because most breeders of dairy cattle do not register females twinned with bulls, as they are so commonly sterile, but as milk production is of little consequence to beef breeders they do not discriminate so closely against these individuals. The total number of twins actually produced is therefore, more nearly represented by the beef registries than by the dairy. Second, these particular breeds were chosen instead of the Shorthorn or Red Polled, because the manner of recording animals is much the same throughout both these registries and the entries are made in such a way as to facilitate the compiling of data for our purposes.

The records used were taken from the first forty-two volumes of the American Hereford Record and the first twenty-six volumes of the American Aberdeen-Angus Association. The former contain the registries of 536,000 individuals and cover a period of years from 1880 to 1916, while the latter contain the entries of 220,500 animals from 1886 to 1916.

The details of entry for each animal vary somewhat in the two breeds, but in general they apply to both sets of Herd Books. The details are as follows: registration number, sex, name of animal, date of its birth, names and numbers of the sire and dam, and names of owner and breeder. If any individual is a twin, triplet, or quadruplet this fact is inserted below the animal's name.

Tabulation and treatment of records

Tabulations were made in order to obtain data on (1) the number of multiple births recorded and (2) the ages of the dams at the time of these births. For (1) records of all individuals designated twin, triplet or quadruplet were assembled from the sixty-eight volumes of the two registries. For (2) it was, first,

necessary to note the dates of birth of all twins, triplets and quadruplets and also the registry numbers of their dams. Then by turning to the latter the dates of birth of the dams were procured. Finally a determination of the interval between the dates of birth of the various twins, triplets, and quadruplets and the dates of birth of their respective dams gave the ages of these dams at the time of calving. These results were next arranged according to the various ages of the dams.

The operations of assembling the data and making the necessary age determinations were independently checked. It was now felt that these data were as reliable as possible, considering that they were based on Herd Book records, in which a small percentage of errors is likely to occur. These may be due to mistakes in reporting, to clerical and typographical inaccuracies, and even to occasional deliberate falsification of facts by breeders. However, since the numbers dealt with here are large, the effect of this small percentage of error is negligible.

The above determinations gave the actual numbers of cows producing twins, triplets and quadruplets at different ages. These numbers are based on records taken from cattle in which the rate of production, record of production, and to a large extent the mortality are controlled by man. That is, the present data deal with a selected population of animals and not with a natural one. It was therefore necessary to consider the number of multiple births occurring among cows of a given age in this selected population, in proportion to the total number of births occurring among all dams of the same ages. In order to do this the ages of all cows at the time of calving were calculated. But since it would have been an enormous task to have made this calculation for the dams of the 750,500 animals recorded, the determinations were made only for every one-hundredth individual registered, such a selection being considered a fair random sample of the entire population. As there were a few cases in which all the desired information was not given, the ages at calving were actually obtained for 7471 cows instead of 7505. The number of dams in each age group was now multiplied by 100 to represent the total population.

The data on multiple births and all births were next tabulated according to the various age groups of the dams and the above-mentioned proportions were obtained. The number of multiple births occurring among cows of a given age per one thousand cows calving at the same age was calculated, the ratio obtained from using a thousand as a basis giving the most convenient numbers for final comparison. Although the general term multiple birth has been employed in the above discussion, as a matter of fact twin births only were taken into consideration in these calculations, the numbers of triplets and quadruplets being too few to make any observable difference in the final results. There were but seven of the former and one of the latter registered in both sets of Herd Books.

Records from Hereford Herd Books

Table 5 shows the results of the compilation of the data on the Hereford breed. The first column gives the ages of the dams from one to thirty-three years and the second column the actual number of recorded multiple births for dams of each age. The data in column three represent the total number of cows calving or the total number of all births occurring at each age, the actual figures obtained having been multipled by one hundred, as discussed in the previous section. Column four gives the number of multiple births per one thousand cows calving at the various ages.

The graphs in figure 1 are based on table 5. Curve 1H, constructed from the data in column two (total number of multiple births recorded), shows a fairly rapid ascent from one to four years and is almost as high at five years, after which it descends very gradually. Graph 2H, representing the total number of cows calving at each age, is similar in general form to 1H, the number of records included here, however, being much larger and the mode falling at three instead of four years. The descent of these curves after three and four years is due to the general tendency among breeders to dispose of older cows, unless they are especially valuable, in order to make way for the younger

ones. As a consequence there are fewer data for older cows. That this is the cause of the low number of multiple births recorded for dams beyond four years is shown by graph 3H, based on column four of the table. These comparative data show a consistent rise in the relative frequency of multiple births

TABLE 5

Data from Hereford Herd books, volumes 1–42

AGE	NUMBER OF MULTIPLE BIRTHS	TOTAL NUMBER OF COWS CALVING	NUMBER OF TWINS PER 1000 COWS CALVING
1	3	3,000	1.00
2	120	71,800	1.67
3	281	89,300	3.14
4	351	79,800	4.39
5	346	64,700	5.34
6	288	53,700	5.36
7	248	44,300	5.59
8	205	35,400	5.79
9	161	26,700	6.02
10	127	20,100	6.31
11	95	14,000	6.78
12	67	9,700	6.90
13	45	6,500	6.92
14	26	3,900	6.66
15	10	2,100	4.76
16	7	1,200	5.83
17	4	600	6.66
18		400	
19	1	200	5.00
21		200	
22		100	
23		100	
29	2		
33		100	
Blanks	42	8,100	
Total.....	2,429	536,000	
Minus blanks.....	42	8,100	
Total.....	2,387	527,900	4.52

up to twelve or thirteen years of age. Therefore the age for highest multiple birth production is not at four but at least as late as twelve or thirteen years. Beyond this age the data are so few that the curve is irregular and has no significance.

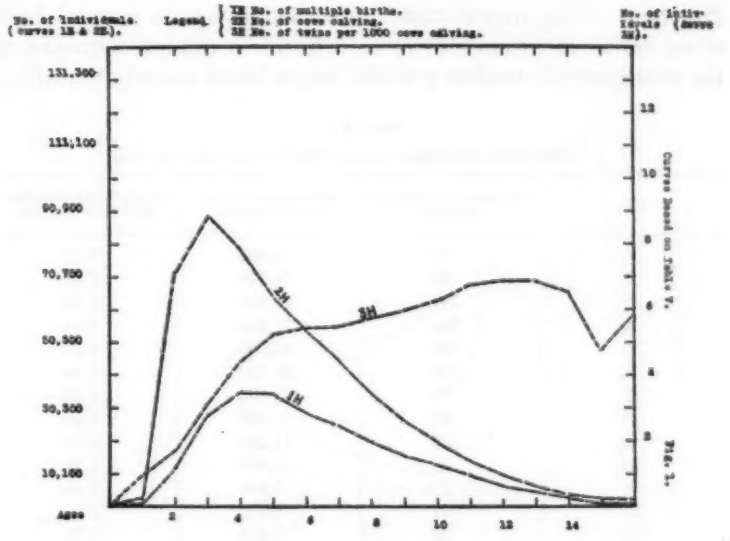

FIG. 1

Records from Aberdeen Angus Herd Books

Data on the Aberdeen-Angus breed, corresponding in material and arrangement to that in table 5, are incorporated in table 6. The total number of records available on the Angus are only about half as many as on the Hereford. Furthermore, the oldest dam reported as calving in this breed was aged twenty-five years, while in the Hereford, records were given for dams up to thirty-three years of age.

Figure 2, based on table 6, is in general similar to figure 1. Curve 1A represents no striking difference from 1H, as the degree of ascent and descent of both graphs is practically the same and the modes of both curves fall at four years. The age at which the largest number of multiple births occurs is accordingly the same for both breeds. Curves 2A and 2H are also similar in general appearance, but the mode of the former occurs at two instead of three years, showing that the largest number of recorded births occurs a year younger for Angus dams than for

Hereford. This means that Angus heifers are in general bred at an earlier age than Herefords, which is a natural sequence of the characteristic tendency of the Angus breed to early maturity.

TABLE 6

Data from Aberdeen-Angus Herd books, volumes 1–26

AGE	NUMBER OF MULTIPLE BIRTHS	TOTAL NUMBER OF COWS CALVING	NUMBER OF TWINS PER 1000 COWS CALVING
1	3	5,800	0.51
2	62	34,300	1.80
3	111	32,800	3.38
4	144	31,200	4.61
5	118	25,000	4.72
6	116	21,300	5.44
7	74	15,900	4.65
8	67	15,200	4.40
9	57	11,200	5.08
10	50	8,500	5.88
11	22	6,200	3.54
12	27	3,700	7.29
13	22	3,800	5.78
14	6	1,900	3.15
15	8	600	13.33
16	5	1,000	5.00
17	2	300	6.66
18	3	300	10.00
19	1	100	10.00
21	2		
22		100	
25	2		
Blanks	25	1,300	
Total.....	927	220,500	
Minus blanks.....	25	1,300	
Total.....	902	219,200	4.11

Curve 3A showing the number of twins per thousand total births in the Angus, resembles very closely the corresponding curve (3H) for the Herefords, but is more irregular beyond nine or ten years owing to the relatively small number of records.

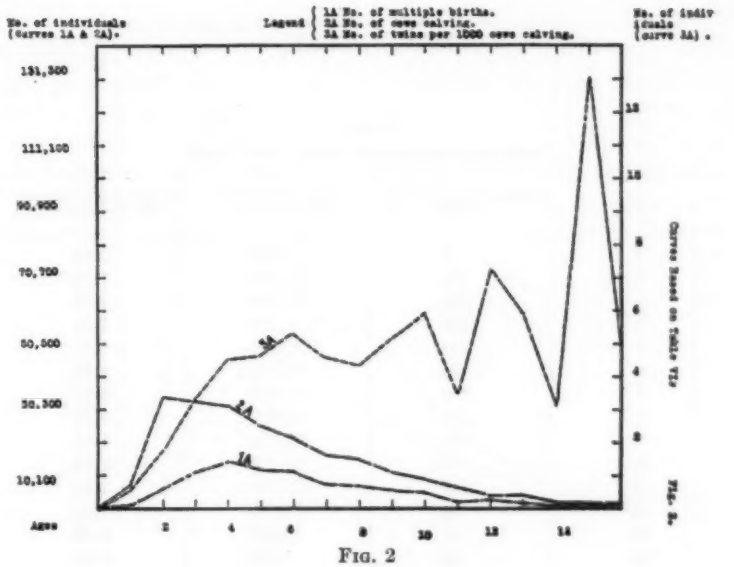

FIG. 2

Combined records of both breeds

Figure 3 is constructed from the data in table 7 made by combining the records of tables 5 and 6. The curves of this figure are the same in general form as the corresponding curves in the two preceding figures, but are smoother because of the larger amount of data upon which they are based. This emphasizes again the fact that the two sets of data, from the two different breeds, exhibit essentially the same features. The significant characteristic of curve 3C is its consistent rise to at least ten or twelve years and, though the data are relatively few and the curve consequently less regular beyond this point, its inclination to maintain about the same level, as may be seen from the data in column four of table 7. At any rate, there is no indication of a decisive falling off in the tendency to produce twins.[5]

[5] Dr. Florence E. Allen fitted curves to the number of cows calving and number of multiple births, as shown in figure 3, according to Pearson's methods given in the Philosophical Transactions, Pt. 1, A, 1895. The curve was fitted to the number of multiple births per 1000 cows calving by taking the ratio of the formulae of the curves 1C and 2C.

TABLE 7

Combined data on both sets of herd books

AGE	NUMBER OF MULTIPLE BIRTHS	TOTAL NUMBER OF COWS CALVING	NUMBER OF TWINS PER 1000 COWS CALVING
1	6	8,800	0.68
2	182	106,100	1.71
3	392	122,100	3.21
4	495	111,000	4.45
5	464	89,700	5.17
6	404	75,000	5.38
7	322	60,200	5.34
8	272	50,600	5.37
9	218	37,900	5.75
10	177	28,600	6.18
11	117	20,200	5.79
12	94	13,400	7.01
13	67	10,300	6.50
14	32	5,800	5.51
15	18	2,700	6.66
16	12	2,200	5.45
17	6	900	6.66
18	3	700	4.28
19	2	300	6.66
21	2	200	10.00
22		200	
23		100	
25	2		
29	2		
33		100	
Blanks	67	9,400	
Total.....	3,356	756,500	
Minus blanks.....	67	9,400	
Total.....	3,289	747,100	4.40

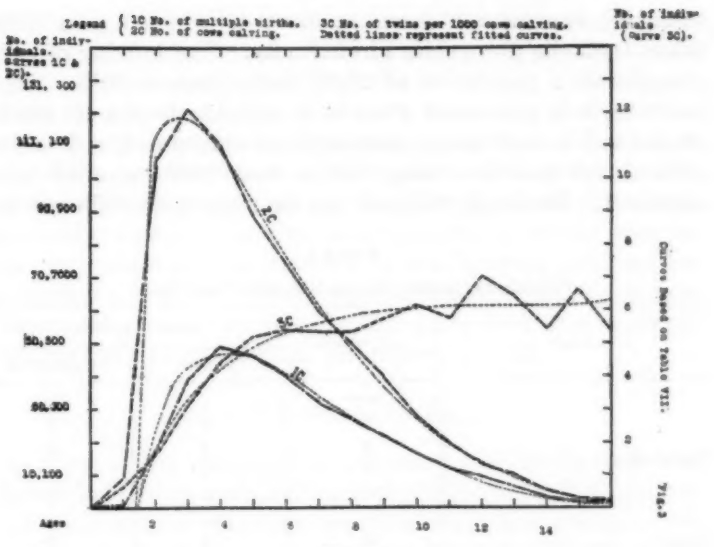

FIG. 3

Triplets and quadruplets in both breeds

As already mentioned only twins were considered in the preceding tables, the numbers of triplets and quadruplets being too few to have an appreciable influence on the results. The recorded triplets and quadruplets, shown in table 8 are given only for the interest attached to the occurrence of more than one or two calves at a single parturition.

A consideration of the frequency of the different orders of multiple births shows that there is one twin birth to every 221 births in the Hereford breed and one to every 243 in the Aberdeen-Angus. For the two breeds combined the twin births are one in 224, or 0.4 per cent of all births. This is considerably lower than Newman's statement (1917, p. 122) that twins in cattle constitute about 2 per cent of the total number of births. The percentage of twinning in man, calculated from the ratio given by Bland (1781) and Duncan (1866), who state that twin births occur once in every 80 deliveries, is 1.25 per cent. The

frequency is greater in sheep, as shown by Plumb's data (1905) taken from the Shropshire Flock Books. The number of twins recorded in a population of 23,037 individuals is 9053. Since, however, it is not stated when both individuals of a set are included and in how many cases only one is recorded, it is impossible to tell just how many births these 9053 recorded twins represent. Obviously it could not be more than 9053 nor less

TABLE 8

Triplets and quadruplets in both sets of herd books

BREED	AGE OF DAM	NUMBER OF CASES	
		Triplets	Quadruplets
	years		
Hereford	2	0	1
	4	2	0
	7	2	0
	10	1	0
Angus	4	2	0
Total	Av. 3.86	7	1

than half that number; in other words the twin births were at least 19.6 per cent of the total births, but not more than 39.2 per cent.[6] The frequency of triplets, as found in the present study of cattle, is as follows: one in every 105,580 births in the Hereford; one in every 109,600 in the Angus; or one in every 106,728 births in the two combined. The percentage of triplets

[6] This uncertainty does not exist in our cattle data, as the records were carefully checked to determine just which recorded twins belonged in sets, so that in this way the actual number of twin births involved was ascertained.

Equation for cows calving, origin at the mode (2C).

$$y = 1212.34\left(1+\frac{x}{1.27648}\right)^{0.29088}\left(1-\frac{x}{21.42753}\right)^{4.88284}$$

Equation for twins, origin at the mode (1C).

$$y = 468.45\left(1+\frac{x}{2.78}\right)^{0.8369}\left(1-\frac{x}{22.7117}\right)^{6.8348}$$

and quadruplets in all multiple births is 0.24 per cent, which is decidedly lower than figures found by Cole (1916), who reported seven cases of triplets in 303 multiple births. The true percentage, however, probably is between these two figures, as the herd books do not include all triplets dropped, as for example those which are aborted, while Cole's data were collected without regard to the proportions of the different classes of multiple births and probably include an undue number of triplet records. Only one quadruplet was recorded for the Hereford and none for the Aberdeen-Angus. There were no records on larger numbers of calves dropped at one time, though cows have been reported as giving birth to as many as six fœtuses (p. 272).

MULTIPLE BIRTHS IN SHEEP

As noted in the Summary of Literature (p. 270) the first data collected by Carlyle and McConnel (1902) from the flock records of the University of Wisconsin indicated that the highest percent of twin births in sheep occurred among five year old ewes, while the ater report of Humphrey and Kleinheinz (1907) gave four years as the age at which the highest percent of twins was found. Later records from the same flock have been compiled by the present authors and are combined with the earlier ones in table 9, the arrangement of the data being essentially the same as in the preceding tables of multiple births in cattle, except that twins and triplets are first considered separately and then combined in column four. Twins and triplets were combined in sheep because of the relatively large number of triplets in sheep as compared with cattle. The number of triplets is not so large, however, as to influence the final calculations in any marked degree. This is shown by the frequency distributions in columns 6 and 8, which are essentially similar. Although the present records are considered on a per thousand basis instead of percentage, as was done in the two earlier reports on the same flock, they admit of ready comparison with the latter by a decimal shift of one place.

Figure 4 is based on data in table 9. The graph for the total number of ewes lambing at the different ages (column 5) begins at the highest point, two years, after which there is a consistent and regular decline. The curve for the total number of multiple births (column 4) is somewhat similar, except that the highest point is reached at three years and the drop is slightly more gradual. These two curves merely show that more records are available on two and three year old ewes than on older ones, because of the general custom among sheep raisers to dispose of older individuals. The horizontal dotted line shows the number

TABLE 9

Data from University of Wisconsin flock, 1890–1917

AGE	TWINS	TRIPLETS	TOTAL NUMBER OF MULTIPLE BIRTHS	ALL BIRTHS	TWINS PER 1000 BIRTHS	TRIPLETS PER 1000 BIRTHS	MULTIPLE BIRTHS PER 1000 BIRTHS
2	159	4	163	367	433.24	10.89	444.14
3	156	12	168	284	549.29	42.25	591.54
4	136	13	149	223	609.86	58.29	668.16
5	105	7	112	162	*648.14*	43.20	*691.35*
6	54	9	63	93	580.64	*96.77*	677.41
7	26	2	28	48	541.66	41.66	583.33
8	10	1	11	15	666.66	66.66	733.33
9	2		2	2	1000.00		1000.00
All ages....	648	48	696	1194	542.71	40.20	582.91

of multiple births per one thousand births produced by ewes of all ages or, in other words, the average age at which multiple births occur. The curves representing the number of multiple births per one thousand ewes lambing at each age (twins and triplets combined) is the significant one for our consideration. This shows that according to the present records the greatest number of multiple births is produced by five year old ewes. The four and six year old females also show a high degree of fecundity. After six years, however, there is a sudden drop, which is probably significant since the number of records for seven years is comparatively large. The rise at eight years

may be due to lack of sufficient data for that age and at nine years it is obviously due to this cause, since it is not at all probable that all nine year old ewes would produce twins.

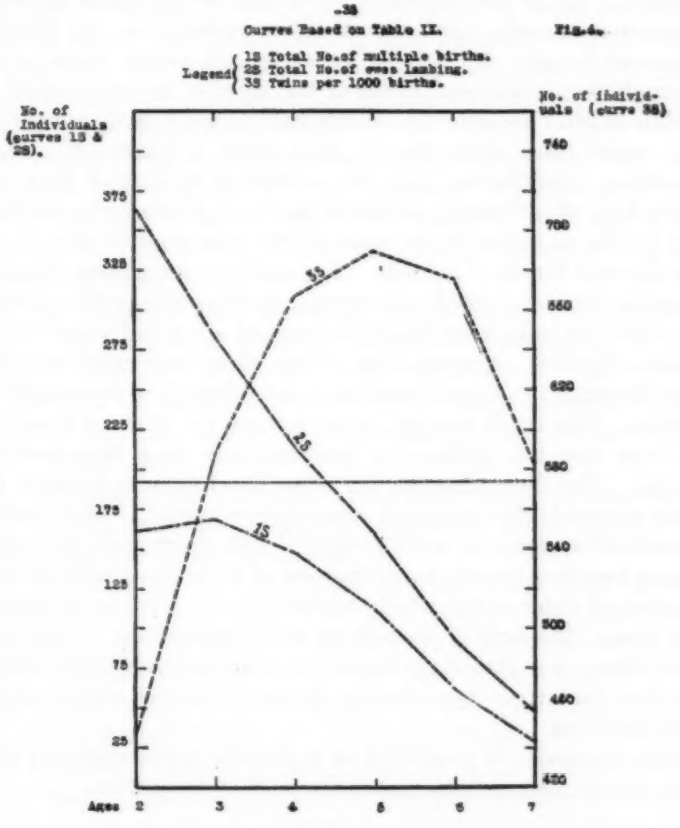

Fig. 4

The combined data on forty-eight sets of triplets are given in column three. As shown in column seven, six year old ewes produce the largest number of triplet sets per thousand births.

DISCUSSION

Observations on fecundity as affected by the age of the female in uniparous animals coincide with similar ones on multiparous animals. All of the instances cited lead to the same general conclusion, namely, that size of litter increases as the female advances in age. In fact, so far as we are aware, there is no contradictory evidence. Some of the records, however, show a decline in litter size after the female has reached a fairly advanced age, while other data fail to show such a regression. This condition, nevertheless, may be attributed to lack of data on individuals of sufficiently advanced ages to indicate such a decline and to the variation in the span of the reproductive periods of the different breeds of animals. For example, some cases relating to swine showed a continued increase in litter size, while others, in which the data were fuller for sows of advanced years, indicated a decline. A comparison of the sheep and cattle records may illustrate the second point in regard to length of reproductive periods. The cattle records, so far as they go, show no decrease in litter size but indicate a comparatively long reproductive period. The sheep data on the other hand show a decrease in litter size and also a shorter reproductive period. This longer reproductive period in cattle combined with the general tendency among breeders toward early disposal of their stock reduces the number of older cows, which might calve in their later years, and hence, the lack of records on such individuals. Although most sheep are also slaughtered at comparatively early ages, the fact that their reproductive period is shorter makes more data available.

This discussion is presented as a possible explanation for the lack of a decrease in litter size in the present cattle data.

SUMMARY

1. The terms fecundity and fertility (and similarly fecundation and fertilization) have commonly been used interchangeably.

2. Fecundity in the female may be *potential* (the sum total of ova capable of being produced by the ovary), *actual* (the ova

actually matured and discharged) and *observed* (the ova of which there is visible evidence, as by the production of eggs or young). The ability to produce offspring is defined by Pearl and Surface as *fertility*, and in mammals this is the same as observed fecundity.

3. Embryological evidence from a number of forms indicates that, in most if not in all cases, actual fecundity is somewhat greater than the observed. The discrepancy is probably not so great, however, but what the number of offspring produced make a fair measure of fecundity.

4. Observations on multiparous animals (rats) show that the frequency of production of litters increases with the age of the female, at least within limits. This point is not capable of determination in the uniparous domesticated animals, at least on the data available.

5. The evidence also indicates, that in animals in general, there is an increase of litter size with the increasing age of the male. This usually goes to a maximum beyond which there is a decline. In the uniparous forms litter size is more commonly expressed in terms of the relative production of multiple births to all births.

6. A review of the literature shows more or less fragmentary records on a considerable variety of animals, which all tend to substantiate the above statements.

7. The data on age of dams at time of giving birth to the 747,100 individuals recorded in the American Hereford and Aberdeen-Angus Herdbooks, volumes 1–42 and 1–26 respectively, show that there is at first a relatively rapid increase in fecundity with the advancing age of the female, followed by a more gradual rise, and without indication of a subsequent decline.

8. From the records of age of dam at birth of 1194 lambs in the flock of the University of Wisconsin, a similar curve was obtained, except that in this case it reached its maximum for dams at five years of age, beyond which there was a decided drop.

9. These findings on cattle and sheep coincide with earlier ones on other animals, since all show a general tendency for older females to produce larger litters than younger ones.

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NOTE: Illinois Bulletin No. 226 (May, 1920) on Variations in Farrow; with Special Reference to the Weight of Pigs, by W. J. Carmichael and John B. Rice, has come into our hands since the present paper went to press. The data consists of records on 708 litters totaling 5774 pigs. The general average litter size is accordingly 8.1. The authors state (p. 72) that there was "a gradual increase in the size of the litter as the sows grew older up to the time they were three years old," after which age the tendency for decrease in litter size became apparent. The litter sizes of sows of one and one and a half years old are 7.5 and for two year olds or over 8.6.

AN UNUSUAL OUTBREAK OF ROPY MILK

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INTRODUCTION

The slimy or ropy fermentation is the abnormal change in milk that is most frequently reported to the dairy section of the Iowa agricultural experiment station. A considerable number of outbreaks of this kind have been studied from the standpoint of the causative organism[1] and *Bact. viscosum* has been isolated more than any other type. Recently an organism not previously encountered has been found as the cause of ropy milk and the results obtained in its study are given below.

HISTORY OF THE TROUBLE

An outbreak of ropy milk was reported to the dairy section from one of the smaller towns in the state. The ropy condition was observed only in the supply of one dealer and seemed to be present mainly in the cream, the usual complaint being that, when the cap was removed, cream adhered to it and was drawn out into strings several inches long. Two samples were sent to the laboratory for examination with the following designations:

Sample 1. Ropy cream.

Sample 2. From same dairy as sample 1 two days later. Shows but little "ropy."

It was necessary to send the samples without ice and they arrived at the laboratory in a sour condition. Transfers made into litmus milk and held at room temperature coagulated rapidly without any development of ropiness, while others maintained at 10°C. showed nothing but a reduction of the litmus even after several days. Infusion agar plates, poured with the original

[1]See Buchanan and Hammer, Res. Bul. 22. Iowa Agr. Expt. Sta. 1915, for a review of the literature on ropy milk and an account of some of the earlier outbreaks studied at the Iowa Station.

material as soon as it arrived, and incubated at 20°C. showed after two days a considerable number of viscous colonies that somewhat resembled colonies of *Bact. viscosum* but which, when older, were considerably more opaque than colonies of this organism. Sample 1 showed a much larger percentage of viscous colonies than did sample 2. Litmus milk tubes inoculated with some of the viscous colonies and held over night at room temperature showed an extremely ropy condition without any evident change in the reaction of the milk. With an increase of twenty-four hours in the age of these cultures there was, however, a pronounced digestion of the milk and it seemed evident that the organism was not the usual type (*Bact. viscosum*) causing ropiness in Iowa. This idea was substantiated by a microscopic examination which showed that the organism was a Gram + coccus.

Shortly after the above mentioned outbreak was reported, a letter, telling of trouble from ropy milk, and a sample were received from a producer located almost 100 miles from the city in which the outbreak had occurred. The sample was not ropy and still sweet on arrival and was plated on infusion agar and the plates incubated at 20°C. Viscous colonies quickly developed and these when inoculated into litmus milk and held at room temperature produced a ropy condition without any noticeable change in the reaction and, later, an extensive digestion of the milk. A microscopic examination showed the organism to be a Gram + coccus, and its similiarity to the organism isolated from the outbreak referred to above is thus evident.

The isolation from two widely separated localities of ropy milk organisms that a preliminary examination showed to be the same seemed especially important in view of the fact that this type had not previously been encountered in a considerable number of outbreaks of ropy milk that had been investigated. Inquiry showed, however, that the milk causing the outbreak was shipped in by the dairymen sending in the sample from the second source, and the relationship between the two complaints was thus cleared up.

The unusual character of the organism suggested an attempt to determine its source. Although at the time such an attempt was made ropiness had not been observed for some time, the producer collected samples in various ways in sterile bottles sent from the laboratory. The plates poured from the samples on arrival and incubated at room temperature did not show viscous colonies, although the number of colonies per plate was low. The original samples failed to develop a ropy condition when held at room temperature. After the samples had been at room temperature for about two days they were plated again and, after room temperature incubation, plates from two of the samples showed a very few ropy colonies. Both of the samples were drawn directly from the udder into sterile bottles, the udder having been previously washed with a disinfecting solution in the case of one sample, but not in the case of the other. Samples taken from the milking machine which was regularly used on the farm and in various other ways failed to show ropy organisms with the methods of examination used. The data obtained indicate that the organisms may have been gaining entrance to the milk in the udder; the results, however, are not at all conclusive because the examination was made at a time when ropiness was not developing in the milk held under practical conditions and it is entirely possible that the organisms may have gained entrance to the udder subsequent to their appearance in the general milk supply. It is rather surprising that the ropy organisms were not isolated from all of the samples instead of only those drawn from the udder in such a way as to largely or entirely eliminate outside contamination; this may, however, have been due to an overgrowth with other organisms in the case of the samples not drawn so as to limit contamination from external sources.

The first report of the ropy condition under consideration was received early in January, 1920. The time of year suggests an unusual source of contamination, since most of the outbreaks of ropy milk occur during the warmer months when infection from surface water, etc., is more likely to occur, and favors the idea of the infection of the milk from the interior of the udder.

If the udder was the true source of the organisms causing the abnormality, it must have been eliminating them in larger numbers than it was at the time the samples secured in various ways were examined or, what is very improbable, the milk must have been held under conditions more favorable for the growth of the ropy organism than those under which the samples were held.

The ropy organism isolated produced a ropy condition in whole milk whether unheated, pasteurized, or sterilized, in a considerable number of trials at both 20° and 37°C. In the unheated and pasteurized whole milk the ropiness was largely confined to the cream layer and even when the cream layer was very ropy the milk below was of a normal consistency; at both temperatures after the first appearance of ropiness the condition increased for a time and then decreased, the decrease apparently being hastened by the development of acid. With sterile whole milk the ropiness was more pronounced in the cream layer, which was very narrow as a result of the heating, but the milk below also showed this condition. The ropiness seemed to increase and then decrease in the sterile milk in much the same way as in the unsterile. With all the different lots of whole milk the ropiness was greater at 20°C. than at 37°C.

In sterile skimmed milk, ropiness developed rapidly in the upper layers and was already evident in test tubes in six to eight hours at 20°C. At 37°C. ropiness also developed rapidly but was never as pronounced as at 20°C. There seemed to be an increase and then a decrease in the ropiness although in tubes at 20°C. the material was still ropy when digestion was practically complete.

As has already been stated, the ropy organism rapidly digested sterile skimmed milk in tubes held at room temperature. At 37°C., however, inoculated sterile skimmed milk did not undergo a rapid digestion, but soon showed a definite coagulation which acid determinations proved was not due to the acidity developed; on standing there was some increase in acidity but even after considerable periods of time the acidity was not high enough to cause coagulation. For example, in a small bottle of inoculated sterile skimmed milk the acidity after twenty-four hours, which

was about the time of coagulation, was 0.31 per cent, calculated as lactic acid, while after nineteen days it was 0.47 per cent.

There are apparently factors other than temperature which influence the rate of digestion. Tubes of sterile whole milk which, as a result of the fat layer, presumably had a smaller air supply than did tubes of skimmed milk showed a much slower digestion than did the latter. Containers having a deeper layer of skimmed milk than tubes also seemed to show a slower digestion.

Since the ropiness occurred at a time when it would be expected that the milk was being held at a fairly low temperature, the ability of the organism to produce ropiness in milk at approximately 10°C. was tried. A ropy condition developed in inoculated sterile milk held at this temperature but comparative tests with a culture of *Bact. viscosum* showed that this organism produced ropiness in milk in a shorter time at approximately 10°C. than did the coccus form. Both organisms, however, eventually produced a very ropy condition.

The organisms usually responsible for ropiness in milk are definite capsule formers, at least in young cultures. With the coccus form isolated, capsules were not observed, but it seemed that a product that was viscous was being secreted by the organisms, since strands of well-stained material were commonly found stringing out from the cells and often running from one cell to another. The microscopic pictures obtained suggested that material quite like the capsular material of *Bact. viscosum* was secreted but was rapidly taken up by the surrounding medium instead of being retained near the cell in a definite capsule. The situation with the coccus form seems to be much like that with such typical capsule formers as *Bact. viscosum* at the time of very pronounced ropiness, when the capsular material is strung out through the medium instead of being retained in a definite form.

The organism responsible for the outbreak of ropy milk was studied morphologically, culturally, and bio-chemically and the data secured were compared with the published descriptions of the organisms reported as causing ropiness in milk. The comparison indicates that the organism isolated is closely related

to *M. mucofaciens* of Thöni and Thaysen[2] which was isolated from ropy milk in Berne and is also somewhat like *M. Freudenreichii* of Guillebeau,[3] an organism that is commonly the cause of ropy milk in Switzerland. Perhaps the most striking difference between the isolated type and *M. mucofaciens* is the color-producing power of the latter; while this may not be an important difference, the work of Winslow, Rothberg and Parsons[4] indicates that color production is definite and constant among the white and orange cocci, and accordingly the organism isolated is considered to be an undescribed species and the name *Staph. cremoris- viscosi* is proposed for it.

DESCRIPTION OF STAPH. CREMORIS-VISCOSI

Morphology

Form. The organism was definitely spherical.

Size. The organism showed but little variation in size on different media and at various ages. The average cell had a diameter of about 0.9 to 1 micron and the extremes varied from about 0.7 to 1.2 microns.

Arrangement. The organisms were irregularly arranged with isolated cells very common in all the usual media.

Motility. Motility was not observed in young bouillon cultures held at either 20° or 37°C.

Staining reaction. The organism stained readily with the ordinary stain and was definitely Gram + with only an occasional Gram − cell even in old cultures.

Spore formation. Spores were not observed and the organisms taken from cultures on various media and at different ages failed to resist 80°C. for ten minutes.

Cultural characteristics

Beef infusion agar slope. After twenty-four hours at room temperature or 37°C. there was a very heavy, white, viscous growth, slightly raised and with a smooth edge. There was but little change with an increase in age.

[2] Centbl. f. Bakt., Abt. 2, xxxvi, 359.
[3] See Orla-Jensen, Die Bakt. in der Milchwirtschaft, 1913, 79.
[4] Jour. Bact., 1920, v, 145.

Beef extract agar slope. Growth on beef extract agar was of essentially the same character as that on beef infusion agar but was slower.

Whey agar slope. On whey agar, growth was much slower than on beef infusion agar but eventually was of the same general type.

Beef infusion agar stab. Beef infusion agar stabs gave a growth along the line of puncture that at first had the appearance of closely aggregated colonies and later showed tiny projections with rounded ends. There was a heavy, white, viscous surface growth that was raised and had a smooth edge. The type of growth was the same at both room temperature and 37°C.

Beef extract agar stab. Growth was of the same general type as that in infusion agar stabs.

Whey agar stab. Growth was essentially the same as that in infusion agar stabs, but considerably slower.

Beef infusion agar plate colonies. On beef infusion agar plates colonies were evident after twenty-four hours at room temperature and after forty-eight hours were well differentiated into surface and sub-surface colonies. The surface colonies were large, round, viscous, white, very opaque, raised and smooth-edged while the sub-surface colonies were smaller, round to ellipsoidal, viscous, white, compact and smooth-edged; with an increased incubation there was an increase in the size of the colonies until those at the surface were from 2 to 3 mm. in diameter and those beneath the surface were up to 1 mm. in their longest dimension. At 37°C. the development of colonies on beef infusion agar was essentially the same as at room temperature.

Whey gelatine stab. Growth developed along the stab and then liquefaction began after several days at 20°C. Liquefaction proceeded only slowly and was greater at the surface than down in the medium; the liquefied portion of the gelatine showed a sediment. The entire medium was usually not liquefied even after two months' incubation.

Bouillons. Plain bouillon and bouillons containing various fermentable materials showed a turbidity and slight sediment in twenty-four to forty-eight hours at room temperature or 37°C. With age the turbidity cleared and the sediment increased. The sediment seemed to be viscous and was drawn out into threads on agitation.

Potato. A white, moist, somewhat viscous, spreading growth occurred on potato at both room temperature and 37°C. but development was slow.

Dunham's solution. After 24 hours at room temperature or 37°C. there was a turbidity and slight sediment. With an increase in age there was a greater turbidity and an increased sediment and later the turbidity cleared up leaving only a sediment. At all stages, the sediment was viscous and when brought to the surface could be drawn out into threads.

Uschinsky's solution. There was no evidence of growth at either room temperature or 37°C. even after long periods of incubation.

Plain milk. At room temperature plain skimmed milk quickly became ropy and then digested, the digestion in tubes being almost complete in three or four days. When fat was present digestion was much slower. At 37°C. plain milk became ropy and then soon coagulated with apparently some digestion or at least an expression of whey.

Litmus milk. The changes were essentially the same as in plain milk except that the litmus was reddened in the milk digested at room temperature while it was reduced in the milk coagulated at 37°C.

Peptone (1 per cent) milk. Growth was essentially the same as in milk without peptone.

Peptone (1 per cent) litmus milk. Growth was essentially the same as in litmus milk without peptone.

Bio-chemical features

Gas production. No evidence of gas production was observed in milk or in bouillons containing glycerol, fructose, galactose, glucose, lactose, maltose, sucrose, mannitol, salicin, raffinose, inulin or starch.

Acid production. In milk there was a slight increase in acidity, the maximum acidity observed being 0.47 per cent, calculated as lactic acid. In bouillons containing the various fermentable materials listed above there was very little if any acid development, 0.5 per cent N/1 acid being the maximum found.

Indol production. Indol was not detected after various incubation periods at room temperature and 37°C.

Oxygen relation. The organism was definitely aerobic.

SUMMARY

An outbreak of ropy milk occurring in Iowa, that was unusual in the time of year it appeared, was studied and found to be due to an organism quite different than the type usually responsible

for ropy milk. The organism, which was studied morphologically, culturally, and bio-chemically, was closely related to *M. mucofaciens* (Thöni and Thaysen) and was also somewhat like *M. Freudenreichii* (Guillebeau) but showed characters that made it seem desirable to designate it as a new species, *Staph. cremoris-viscosi*. Some time after the disappearance of the trouble the causative organism was found in the udders of cows in the producing herd. It was impossible, however, to tell whether the invasion of the udders preceded or followed the outbreak of ropy milk; in either case conditions were different when the milk carefully drawn from the udders was examined than when the outbreak occurred since when the examination was made the milk was no longer developing ropiness.

A COMPARATIVE STUDY OF CORN SILAGE IN CONCRETE AND STAVE SILOS[1]

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INTRODUCTION

Owing to the continual increase in cost of feed for dairy cattle, silos are becoming more and more popular. While the wooden or wooden-stave silo is the prevailing type in most sections of the country, concrete silos have come into favor. There seems, however, to be a rather general feeling that the silage produced in concrete silos is inferior in quality to that produced in the stave silo. The purpose of the investigation described in this paper is an attempt to throw some light on this phase of the silo problem.

PREVIOUS INVESTIGATIONS

In 1884, Adolph Mayer, at Wageningen (1), reported the analysis of green maize put in, and maize silage taken out of four silos. In two unlined earth-pit silos containing, at ensiling, respectively 6 tons and 12 tons of maize which remained in the pits four and one-half months and five months, he found a loss of 40 per cent and 37 per cent respectively in food value. By ensiling 12 tons of maize for three months in a double-walled ice house he obtained an 18 per cent loss of food value. Using a cement silo with a roof he found a loss, after six and one-half months, of 36 per cent of the food value of the 6½ tons of maize ensiled.

In 1914 at the Iowa Agricultural Experiment Station, Neidig (2) used three types of round silos, brick, wood-stave, and concrete, having a capacity of 83 tons, 131 tons, and 138 tons, respectively. He decided, after careful study of the results from

[1] Published by permission of the Secretary of Agriculture.

the three silos, that the type of silo has no appreciable effect upon the keeping quality of the silage. He stated among his conclusions:

> The results show that no differences were noted in the chemical changes in the three silos which could be attributed to the effect of the different types of building materials upon the process of silage formation. It is readily seen that approximately the same results are obtained in the temperature observations, gas analysis, and volatile and non-volatile acids of silage from the three silos. The only differences noted are differences in the quantity; the ratio of chemical substances is very nearly the same for each silo.

He stated further in discussing the relative merits of the different types of silos

> The chief factors necessary for the production of good silage are, therefore; smooth, air-tight walls, corn in the right state of maturity, the proper amount of moisture, and carefulness in filling.

During 1913 and 1915, Eckles, Oshel, and Magruder (3) studied temperature changes and chemical changes in a number of types of farm silos, experimental silos, and experimental cans, including two concrete silos and three stave silos. The concrete silos had a capacity of 168 tons each and the stave silos had capacities of 190 tons for one and about 150 tons each for the other two.

The authors concluded:

> The temperature in the silage in the early stage is influenced to some extent by the temperature of the atmosphere at the time of filling and of the water used, if any.
>
> The material used in construction of the silo has but little, if any, influence upon the temperature of the silage.
>
> Analyses of silage from the wall and center of silos of various types of construction showed no difference in composition due to the material used in the construction of the silo.

PRESENT INVESTIGATION

Silos used

The silos used in the investigation were located at the Dairy Division Experimental Farm, Beltsville, Maryland. They held approximately 150 tons of silage each. They were built side by side and consequently were exposed to practically identical weather conditions with the exception that the stave silo was situated directly south of the concrete one and consequently was somewhat more sheltered from northerly winds. The stave silo was also exposed to more heat of the sun.

Temperatures of silos

Temperatures were taken by means of electrical thermometers buried in the silage, with cables leading to temperature indicators where the readings were taken at regular intervals. Three of these thermometers were used in the stave silo and three in the concrete. They were buried at about the same distance from the top in each silo, so that the pressure of silage would be approximately the same at the point at which each of the thermometers was buried. One thermometer was 3 inches from the wall, another 18 inches, and the third in the center of each of the silos. A mercury thermometer was used in taking the temperature of the atmosphere at the time the readings were taken.

Changes in chemical composition

In order to determine the factor of chemical composition a sample of cut corn was analyzed at the time of filling and a sample of the silage after fermentation had ceased. The plan was to run into the silo a considerable quantity of cut corn, thoroughly mix it on a piece of canvas, take a sample for analysis, and fill a number of sacks with a known weight of the cut corn for burial at certain distances from the silo wall. These sacks were made of cheesecloth and a sack was buried at the side of each thermometer so that the centers of the sacks were the same distance from the wall as were the thermometers. In order to get these

sacks buried at the proper places a metal form 6 by 12 by 18 inches and open at both ends was used, into which the sacks were tightly pressed. Cut corn of the same lot from which the sacks were filled was used to fill in around the forms. The forms were then removed. As the sacks were at the same level as the thermometers and the same distance from the wall the temperature as shown by the thermometers could be used in determining the effect of different temperatures upon the chemical composition of the silage contained in the sacks. These samples were removed for analysis as soon as the silage was fed down to the place where they were located. Analyses of both the original and fermented materials were made for kind and amount of acids, water, ash, crude fiiber, total nitrogen, albuminoid nitrogen, ether extract, and nitrogen free extract.

Quality of the silage

The quality of the silage was judged by the appearance, odor, and palatability when fed to cows. It was originally planned to conduct an actual feeding trial to determine the relative nutritive value of the two classes of silage in case the chemical composition and the quality of the silage indicated the advisability of so doing. As no marked difference was observed in the quality of the silage made in the two silos it was decided not to conduct such a feeding experiment.

Methods of analysis

The material on reaching the laboratory was first thoroughly mixed. One kilogram of the mixed material was dried in a steam drying closet at from 50° to 60°C. to constant weight. It was then exposed to the air in the laboratory for a few days, the final weight being taken to represent the air-dry condition. The air-dry material was ground in a power grinder to a fine powder suitable for analysis. Another part of the fresh material was pulped in a power meat grinder, thoroughly mixed, and samples taken immediately for the nitrogen and volatile-acids determinations.

The volatile acids were determined by the method of Duclaux. All other determinations were made according to the methods of the Association of Official Agricultural Chemists.

Two years were covered by the investigation. As the silos were being filled the first season, a breakdown in the cutting machinery after the sacks were placed caused an interruption. The corn which was put into the upper parts of the silos was consequently considerably more mature than that put into the lower parts and it was considered necessary to water both silos. Since both received the same treatment it is not thought that this interfered with the comparative results.

The sacks were placed in both silos, the first season, on October 7. They were removed between March 7 and March 14, having been in the silos 151 to 158 days The sacks were placed in the silos the second season on September 26. Owing to feeding conditions at the farm the level of the sacks was reached much earlier than in the first season. They were removed between October 22 and October 29, having been in the silos 26 to 33 days.

Results of analyses

The following tables give the chemical results on the material as it was ensiled and as it was removed, and also give the temperatures.

The temperatures. For the greater part of the first season temperature readings were taken each day at 6 p.m. Toward the last of the season they were taken less frequently. During the second season, which extended but a little more than a month, the readings were taken at 5 p.m. each day, with one or two exceptions. Unabridged tables containing these data would take up too much space to be presented here. The tables which follow contain figures representing average daily temperatures for each week of the active ensiling period and it is believed that they will serve as well as the much more cumbersome ones giving the daily temperatures.

In comparing the temperatures of the two silos it should be borne in mind that the stave silo was situated directly south of

the concrete silo and received more or less protection from northerly winds and more heat from the sun. This will account, perhaps, for the somewhat lower temperatures in the concrete silo.

SUMMARY AND CONCLUSIONS

Two 150-ton silos, one wood-stave and one concrete, were used in the investigation. They stood side by side and were

TABLE 1

Chemical composition of material when placed in both silos and when removed; first season

CONSTITUENT	GREEN MATERIAL WHEN PUT INTO SILO	SAMPLE NO. 1 STAVE SILO, NEXT TO WALL	SAMPLE NO. 2 STAVE SILO, 18 INCHES FROM WALL	SAMPLE NO. 3 STAVE SILO, CENTER	SAMPLE NO. 4 CONCRETE SILO, NEXT TO WALL	SAMPLE NO. 5 CONCRETE SILO, 18 INCHES FROM WALL	SAMPLE NO. 6 CONCRETE SILO, CENTER
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Moisture	81.71	80.45	81.95	80.43	82.02	83.23	81.69
Ether extract	0.75	0.68	0.80	0.78	0.74	0.64	0.59
Total nitrogen	0.317	0.261	0.262	0.269	0.259	0.256	0.290
Albumin nitrogen	0.256	0.204	0.170	0.178	0.171	0.149	0.168
Crude fiber	6.07	5.30	4.94	4.57	4.37	4.54	4.41
Furfurol	2.09	2.27	1.93	2.15	1.91	1.81	1.94
Starch	3.08	3.18	3.25	3.63	3.12	2.95	3.08
Total sugars as dextrose	0.37	0.025	None	None	0.055	None	None
Invert sugars as dextrose	0.04	None	None	None		None	None
Ash	1.36	1.26	1.31	1.43	1.32	1.18	1.17
		cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
Volatile acids in 100 grams*		72.36	124.5	115.0	99.97	121.9	149.0

* These figures refer to the quantity of $\frac{N}{10}$ $Ba(OH)_2$ solution required to neutralize the total volatile acids in 100 grams of pulp.

exposed to the same temperature and weather conditions, with the exception that the stave silo, which was located to the south of the concrete silo, received some protection from northerly winds and more heat from the sun.

When the silos were about half filled, sacks of carefully mixed cut corn were buried near the wall, 18 inches from the wall, and in the center of each silo. Temperatures were taken of the

TABLE 2

Chemical composition of material when placed in both silos and when removed; second season

CONSTITUENT	GREEN MATERIAL WHEN PUT INTO SILO	SAMPLE NO. 1 STAVE SILO, NEXT TO WALL	SAMPLE NO. 2 STAVE SILO, 18 INCHES FROM WALL	SAMPLE NO. 3 STAVE SILO, CENTER	SAMPLE NO. 4 CONCRETE SILO, NEXT TO WALL	SAMPLE NO. 5 CONCRETE SILO, 18 INCHES FROM WALL	SAMPLE NO. 6 CONCRETE SILO, CENTER
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Moisture	78.09	79.18	78.00	78.34	78.50	78.60	77.48
Ether extract	0.29	0.47	0.47	0.43	0.53	0.46	0.50
Total nitrogen	0.445	0.427	0.408	0.421	0.435	0.435	0.421
Albumin nitrogen	0.424	0.381	0.352	0.368	0.381	0.356	0.370
Crude fiber	4.97	4.23	4.40	4.24	4.28	4.41	4.83
Furfurol	2.49	2.15	2.25	2.20	2.30	2.22	2.12
Total sugars as dextrose	1.65	0.16	0.26	0.69	0.18	0.43	0.39
Invert sugars as dextrose	0.77	None	None	None	None	None	None
Ash	0.94	1.02	0.94	0.90	1.01	0.97	0.96
		cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
Volatile acids in 100 grams ground pulp*		67.1	56.24	56.3	64.7	64.5	61.0

* These figures refer to the quantity of $\frac{N}{10}$ $Ba(OH)_2$ solution required to neutralize the total volatile acids in 100 grams of pulp.

TABLE 3

Volatile acids in silage from the two silos

ACID	SAMPLE NO. 1 STAVE SILO, NEXT TO WALL	SAMPLE NO. 2 STAVE SILO, 18 INCHES FROM WALL	SAMPLE NO. 3 STAVE SILO, CENTER	SAMPLE NO. 4 CONCRETE SILO, NEXT TO WALL	SAMPLE NO. 5 CONCRETE SILO, 18 INCHES FROM WALL	SAMPLE NO. 6 CONCRETE SILO, CENTER
First season						
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Acetic acid	1.84	3.49	2.72	2.47	3.73	2.92
Propionic acid	0.24	0.41	0.38	0.50	0.37	0.24
Second season						
Acetic acid	1.54	1.26	1.19	1.39	1.49	1.35
Propionic acid	0.38	0.16	0.19	0.22	0.08	0.05
Formic acid						0.04

outside air and of the silage at the points where the sacks were buried in each silo. The silage in the sacks was subjected to a rather complete chemical analysis, including the volatile acids. Neither the temperatures nor the chemical analysis revealed any marked difference in the quality of the silage that could be ascribed to the material used in the construction of the silo. Cows ate the silage from both silos with the same avidity.

TABLE 4

Mean temperatures of the air and of the silage at three different points in each silo

WEEK ENDED	AIR	SAMPLE NO. 1 STAVE SILO, NEXT TO WALL	SAMPLE NO. 2 STAVE SILO, 18 INCHES FROM WALL	SAMPLE NO. 3 STAVE SILO, CENTER	SAMPLE NO. 4 CONCRETE SILO, NEXT TO WALL	SAMPLE NO. 5 CONCRETE SILO, 18 INCHES FROM WALL	SAMPLE NO. 6 CONCRETE SILO, CENTER
First season							
	deg. C.	*deg. C.*	*deg. C.*	*deg. C.*	*deg. C.*	*deg. C.*	*deg. C.*
October 13.....	21.3	25.3	31.8	33.0	21.2	26.8	28.6
October 20.....	16.6	23.0	34.1	37.3	18.6	28.3	32.5
October 27.....	14.5	20.2	30.6	36.2	16.4	26.3	34.8
November 3.....	16.0	18.0	27.6	35.3	14.1	24.5	35.6
November 10.....	14.6	15.4	25.3	34.7	12.3	22.5	34.7
November 17.....	13.5	14.8	23.6	34.1	12.1	20.9	33.8
November 23.....	13.9	12.2	22.2	33.4	8.9	19.4	32.5
December 1.....	5.5	9.4	20.3	32.5	6.1	17.3	30.8
December 7.....	8.7	10.8	18.3	31.3	8.7	15.3	29.0
December 14.....	2.7	7.5	17.7	30.7	4.5	14.5	27.7
December 21.....	7.1	7.1	16.0	29.4	4.0	12.6	26.3
Second season							
October 3.....	21.6	28.7	34.5	35.2	23.1	29.1	29.2
October 10.....	22.3	24.5	32.6	37.0	19.9	27.1	29.1
October 17.....	18.7	21.7	30.3	37.0	16.9	24.7	28.5
October 24.....	14.0	20.5	28.9	37.3	13.8	22.6	28.3

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HOW DO BACTERIA GET INTO MILK AT THE FARM AND HOW MAY THEIR NUMBER BE REDUCED?

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The high germ content of city milk supplies has been the occasion of much alarm for the past thirty years. Many cities (1) have attempted to meet the situation by declaring that milk containing more than some definite maximum of bacterial life should be excluded from the retail market.

About twenty years ago there began in this country a series of fruitful attempts (2) to find the actual sources of bacterial life in milk. While there has been a general and growing interest in this line of studies the work has been most intensively pushed at the New York Agricultural Experiment Station, at the Federal Dairy Division, and at the University of Illinois.

But little study was needed to show that the bacteria present in the milk as it reached the city plant were due to two distinct sources; first, the seeding with germ life which the milk receives at various stages in its journey, and second, the growth which takes place after the germs are seeded into the milk.

It has long been known that for some time after the milk is drawn from the cow the germ content ordinarily decreases (3), the extent and duration of this decrease depending upon a number of factors. Gradually the multiplication of some of the germs which have fallen into the milk compensates for the decrease of the others. As a result of the action of various factors, the germ content of the morning's milk as it is delivered from the farm to the bottling plant or the shipping station ordinarily represents little more than the number of bacteria seeded into the milk.

Milk companies in increasing numbers are determining the germ content of the milk as it comes to them. While their

findings are subject to fairly wide variations at any given plant and there are also variations in different parts of the country, 50,000 bacteria per cubic centimeter may be taken as a very conservative figure for the bacterial content of morning's milk as delivered at the shipping station or bottling plant during warm weather.

The problem of the dairy bacteriologist is first to find the sources of this large number of bacteria which get into milk and second, to find practical means by which the introduction of germ life can be markedly reduced. At the beginning of the studies concerning the sources of the bacteria added to milk, there was a general feeling that the character and condition of the barn was mainly the responsible factor. This feeling found expression quite frequently in summary orders from health departments to milk producers either to provide better barns or quit furnishing milk for the municipal supply.

Investigation soon made it evident that the construction of the barn (4), within any reasonable limits, exerted little or no influence upon the germ content of the milk. It was found further that while many of the ordinary barn practices exerted a measurable influence upon the amount of germ life in the milk (5) the magnitude of those influences was plainly secondary. Perhaps their most constructive contribution consisted in pointing out that at least 80 per cent of the germ life getting into milk comes ordinarily from the utensils (6) in which the milk is handled and that among the utensils used at the farm the milk can is ordinarily the major source of germ life.

Having shown that the milk cans, and not the barns, are mainly responsible for the large amount of germ life added to the milk at the farm, the next problem was to find practical means of reducing the germ life in the milk cans. Data were presented which indicated that the germ content of cans washed at the milk plant is progressively reduced when the cans are thoroughly washed, when they are rinsed with clean water, and particularly when they are thoroughly steamed. The data further showed that the amount of germ life in the cans when used later at the farm is largely dependent upon the presence

and area of moist surface persisting in the interior of the cans. These results suggest that if the cans which are now washed and steamed at the milk plants are properly dried before being returned to the milk producers, the germ content of the milk later returned to the milk plants in these same cans will be markedly reduced.

While the solution of the problem of producing milk of low germ content is thus shown to lie largely within the control of the milk plant operator, the fact remains that under present conditions a large part of the cans returned to the producer from the milk plant are returned in a moist condition. Accordingly it is important to know what the milk producer can do with these moist, high germ content cans in order to best prepare them for receiving milk. Moreover, the problem of the most effective, and at the same time the most practical method of handling pails and similar utensils is one constantly present on all farms.

The mechanical washing of pails and cans on dairy farms is both so simple and ordinarily so well done as to need little comment. Moreover, following this washing there is much divergence of practice with regard to subsequent rinsings of the washed utensils with hot water. A large part of the effect of such rinsing upon the germ life has been found to result from the mechanical[1] removal of germ life by the rinse water. The magnitude of this mechanical removal has been measured by experiments in which rinse water at 70°F. was used. The results of such treatment of a considerable number of well-washed 8-gallon cans suggests that more than two billion germs are thereby removed from each can.

A somewhat larger number of germs is mechanically removed by using warmer rinse water, but, as the temperature of the rinse water increases, the measurement of the germs actually removed is complicated by the destructive action of the hot water upon the germs. For example, rinsing with water at 205° to 208°F. undoubtedly removes mechanically more germs than a cooler rinse water will remove, but the destructive action of this high temperature is so great that the number of living germs found in the rinse water rarely exceeds a half billion per can.

[1] This and the following statements regarding results of investigations refer to data given in Ill. Agr. Exp. Sta. Bul., now in press, on Elimination of germs from dairy utensils. I. By rinsing. II. By drying in sun and air.

The destructive effect of hot rinse water upon germ life is much less than is commonly supposed, being much reduced by the rapid cooling of the rinse water through the transfer of its heat to the utensil. Thus when an eight-gallon can at 72°F. is rinsed with a quart of water at 150°F., the temperature of the rinse water falls about 40° within sixty seconds. As the amount of rinse water per can is increased, the drop in temperature is correspondingly reduced, so that when 4 quarts of water at 150°F. are added to a can the temperature drops only about 20°.

When boiling water is applied the destruction of germ life is pronounced, particularly when more than two quarts of rinse water are used per can. Under such circumstances the combined effect of the mechanical removal and the destruction of germ life is such that if the cans were immediately filled with sterile milk the germs remaining in such scalded cans would commonly increase the germ content of the milk only about 100 bacteria per cubic centimeter. Accordingly the fairly common practice of applying scalding water to freshly washed utensils or to moist washed cans returned from the milk plant is to be commended. It should be noted, however, that the pouring of this rinse water from one can to another greatly reduces the effectiveness of this practice, both because the rinse water promptly becomes too cool to exert much killing effect and because it quickly becomes contaminated by the germ life which it mechanically removes from the utensils.

As a method of treating the moist, washed cans which arrive at the farm shortly before they are needed nothing better is available ordinarily than the thorough scalding of the can with hot water. However, when any considerable interval is to elapse before the cans are filled with milk, it is of prime importance that they should be promptly and thoroughly dried.

Observations have been made upon the effect of inverting washed and rinsed cans on a rack in the sun. When the sun is hot and the air dry the cans dry quickly and the germ life in the cans is promptly reduced. Cans which are fairly well rinsed, when thus dried and kept dry, are ordinarily in a condition to

add not more than 100 bacteria per cubic centimeter to the milk with which they are filled. However, during damp or rainy weather, when moisture remains in the cans the germ content holds its own or even multiplies considerably.

A further series of observations was made by adding to sterile cans 10 cc. from a vat of rinse water or wash water and holding the cans twenty-four hours, one half of the cans with the covers on and the other half with their covers removed. Where the covers remained on the cans, the germ life increased between 20 and 3000 fold. Where the covers were removed and the conditions permitted rapid drying, the germ content fell promptly, but where the drying was retarded the amount of germ life remained constant or even increased.

These studies of the source of the bacteria which get into the milk at the farm may be summarized by stating that evidently the milk cans are ordinarily the main factor in seeding the milk, and that during hot weather they normally contribute 30,000 or more bacteria per cubic centimeter to the fresh milk. This high germ content of the milk cans is ordinarily due, not to failure to properly wash and rinse the cans at the milk plant, though improvements at this point are undoubtedly needed in some cases, but primarily to the growth which takes place in the cans after the washing process.

The amount of growth occurring in the milk cans between the time they are washed and the time they are filled with milk depends upon the temperature, the amount of moist area within the cans and the length of time which elapses before the cans are used. Of these factors the moisture of the can is the only one which can be successfully controlled.

A thorough drying of the cans before they leave the milk plant will exert a profound influence upon the germ content of the milk later returned to the plant in these cans.

Where moist cans are returned to the producer he may put them into acceptable condition by thoroughly rinsing them with scalding water. If they are not to be used immediately, they should be promptly and thoroughly dried.

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